Molecular Construction of Sulfonamide Antisense Oligonucleotides

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Abstract

An efficient and scalable synthesis of new oligonucleotide monomers was developed for replacement of the phosphodiester backbone of RNA by a sulfonamide containing backbone to enable construction of sulfonamide antisense oligonucleotides (SaASOs). It was shown that by employing these sulfonamide RNA(SaRNA) monomers it was possible to synthesize oligomers in solution. The properties of a sulfonamide moiety replacement were evaluated by incorporation of a SaRNA-monomer into a DNA strand and performing thermal stability tests of the resulting DNA and RNA-double-strand hybrids. Although the sulfonamide modification caused a decrease in melting temperature (T_m) of both hybrids, it was lower for the sulfonamide containing DNA-RNA hybrid than that for the sulfonamide containing DNA-DNA hybrid.

Introduction

The development of oligonucleotide synthetic methods has led to a plethora of chemical approaches for preparation of derivatives and analogues. As a result, a range of chemically modified antisense oligonucleotides (ASOs) is now available. Over the years further improvements of the synthesis of the nucleotide building blocks and overall efficiency of oligonucleotide syntheses made it possible that sufficient quantities of ASOs became available for clinical use. This has culminated in recent FDA-

approvals of three ASOs for treatment of homozygous familial hypercholesterolemia (Kynamro®),[1] Duchenne muscular dystrophy (Eteplirsen®),[2] and spinal muscular atrophy (Spinraza®).[2] The developments in siRNA and more recently in CRISPR research have sparked a renewed interest into innovative oligonucleotide chemistries.

Excellent reviews have discussed the different important aspects of ASOs.[2-7] Understandably, because nucleic acids have a sugar phosphate backbone, the majority of ASOs are phosphate analogues. Especially prominent are the thiophosphate containing ASOs (including Fomivirsen and Spinraza). Nevertheless, thiophosphate ASO chemistry remains challenging and the introduction of additional phosphor chiral centres leads to a complex mixture of ASOs. However, the development of ASOs completely devoid of a sugar phosphate backbone, peptide nucleic acids (PNAs), in the early nineties, showed that it is possible to design other readily accessible mimics of nucleic acids, with improved properties compared to the existing ASOs.[8] It is therefore somewhat surprising that relatively little attention has been spent on sulfur-based backbone modifications in comparison to phosphorus modifications as these also give rise to tetrahedral geometries. The oxygen atoms attached to a tetrahedral sulfur atom are not charged, so the resulting sulfur-containing linkage will likely contribute to easier penetration into cells as opposed to the negative charge of an oxygen or sulfur atom attached to a tetrahedral phosphorous atom. Furthermore, chemically and metabolically stable bonds to the tetrahedral sulfur atom can be introduced. Lastly, the absence of chirality in the linkers connecting the nucleotide building blocks results in the formation of a single stereoisomer in contrast to the phosphorus-containing linkages, which are present in the therapeutically used ASOs.

The lack of sulfur-containing ASOs (S-ASO) could be due to difficulties in developing a highly efficient oligomerisation chemistry strategy. The realization of efficient syntheses of phosphorus oligonucleotides took several decades and included the breakthrough transition from phosphor chemistry pioneered by Khorana et al.[9] to phosphoramidite chemistry especially advanced by Caruthers et al.[10,11] These impressive developments also set the stage for ASOs with a phosphor(di)amidate or phosphorthioate backbone which are present in the FDA approved ASOs. The

chemistry development of these ASOs and subsequent clinical studies took several years. This at least partly explains the low interest in the development of novel S-ASOs.[8]

Other reasons for the absence of S-ASOs might be the potential properties of several of these derivatives. Bivalent sulfur containing ASOs **1**[12] are likely to be practically insoluble in aqueous solvents. This is likely also the case for sulfone containing ASOs **3**[12-14]. Sulfoxide containing ASOs **2** are probably more polar and therefore better soluble in aqueous solvents, but the sulfoxide moiety is chiral leading to diastereomeric mixtures of ASOs and for this reason may not have been investigated. Sulfonate (**4** and **5**[15]) or sulfate containing ASOs **6** are of course inherently unstable and therefore unsuitable because of their susceptibility towards attack by nucleophiles including abundantly present water (Figure 1).

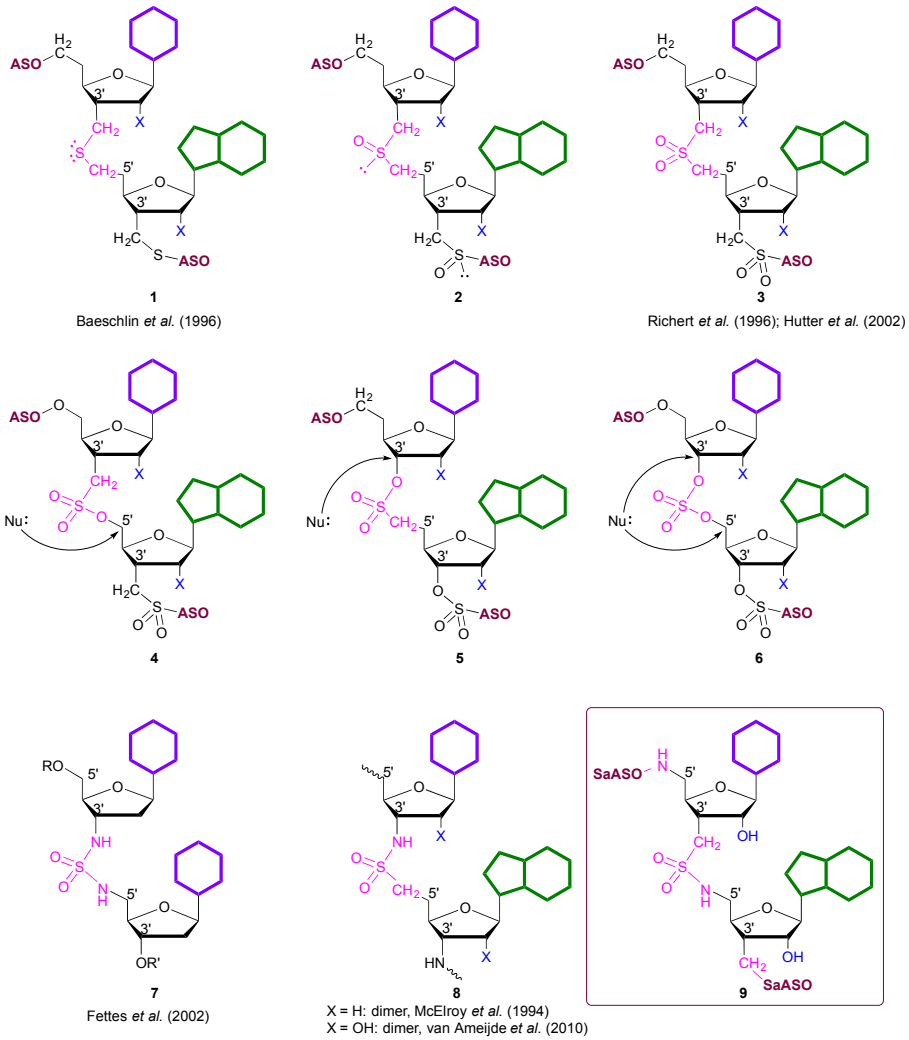


Figure 1. Sulfur containing ASOs. ASO **1**, ASO **3,** ASO **7** and ASOs **8** have been described in the literature; ASOs **4 - 6** are expected to be unstable; ASO **9** is described in this work.

However, sulfur containing ASOs containing the sulfonamide moiety (**8** and **9**) may be attractive, since the sulfonamide moiety is more polar than the sulfone moiety in ASO **3** and the presence of an amide bond hints at a synthetic possibility of repetitive formation so that an ASO containing several sulfonamide moieties should be synthetically accessible. In view of its anticipated polarity the sulfamide moiety in ASO **7**[16] also seemed an interesting alternative.

Two types of sulfonamide containing ASOs (SaASOs) are possible. (Figure 1) In the first type (**8**) the sulfonamide moiety is obtained by coupling of a 3'-amino nucleoside derivatives with a 5'-sulfonyl chloride nucleoside derivative. The introduction of this moiety was first described by Widlanski et al.[17] We have improved the synthetic strategy by development of more convenient nucleoside building blocks in which the amine group was masked as an azide allowing in principle a repetitive cycle of two reactions - (1) unmasking of the azide and (2) coupling of resulting amine and sulfonyl chloride - which can be used for the construction of sulfonamide containing ASOs.[18] Nevertheless, this approach had the disadvantage that a relatively hindered 3'-amino functionality has to act as the nucleophile to form the sulfonamide moiety. The original method of Khorana for synthesis of a phosphate triester suffered from a similar disadvantage in that a secondary 3'-hydroxy functionality had to attack the activated phospho triester derivative (Figure 2). This was later remedied by the development of the phosphoramidite method. Not only is a phosphoramidite derivative much more reactive than any activated phospho triester derivative, but also in the amidite method the 5' -primary- hydroxy functionality is used as a better, less hindered, nucleophile (Figure 2).

Thus, we wished to develop an efficient and scalable synthesis for sulfonamide containing ASOs obtained by reaction of a 5'-amino group containing nucleotide derivative with a suitably activated 3'-sulfonic acid derivative (Figure 2).

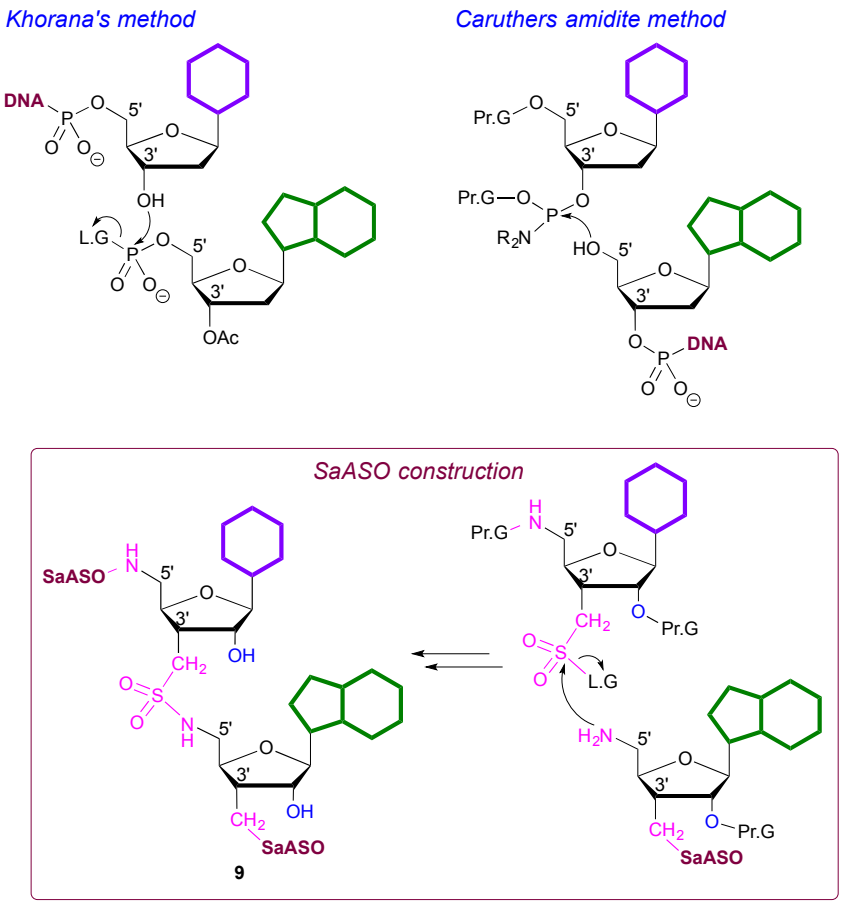


Figure 2. Proposed sulfonamide ASO (SaASO) construction by reaction of a 5'-amino nucleophile with a 3'-sulfur electrophile comparable to the phosphoramidite method of Caruthers and co-workers.[9,10]

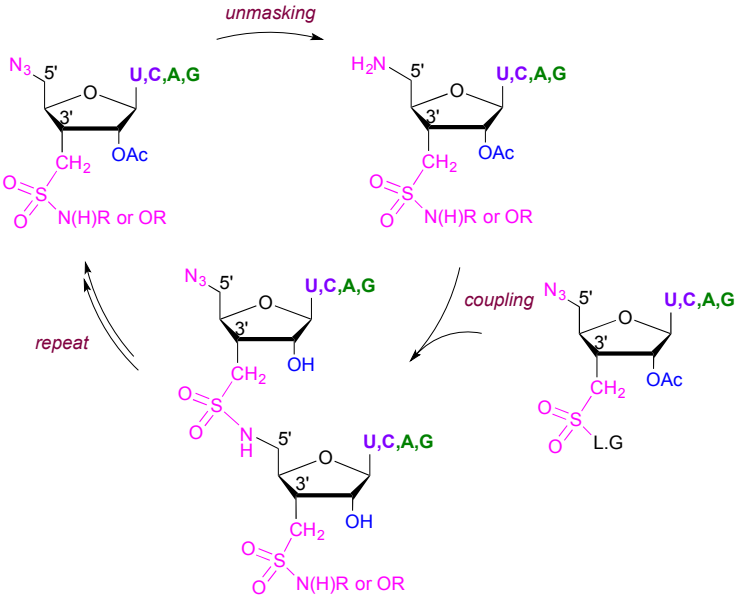
Results and discussion

Preparation of the SaRNA nucleoside synthon

When considering molecular construction of biopolymer mimetics such as sulfonamide containing ASOs, the most important issues are (1) convenient access to their building blocks and (2) requirement of a versatile and efficient coupling strategy for formation of a sulfonamide linkage. Therefore, we wished to achieve versatile access to the building blocks for sulfonamide ribose based oligonucleotides (SaRNA) to realize both aims. It would be beneficial that the devised molecular construction strategy is in principle amenable for translation to a solid phase synthesis protocol.

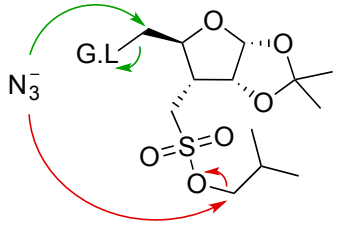
Finally, convenient adaptation of the synthesis to achieve molecular construction of RNA/DNA – SaRNA hybrids was desired. With these requirements, we set out to devise a synthetic strategy.

In our opinion the crucial issue in the molecular construction of SaRNA was the availability of a versatile method for synthesis of the sugar sulfonamide backbone (Figure 1). In order to ultimately couple the nucleotide SaRNA building blocks in a repetitive manner, one needs a readily deprotectable amine as well as a readily activatable sulfonic acid moiety (Figure 2). The azide, serving as a 'masked' amine and a hindered sulfonate ester have been used earlier by us in the preparation of a sulfonamide linkage.[18] In this strategy the 5'-azide is unmasked at every synthesis cycle and the resulting 5'-amine is then coupled to 3'-activated sulfonic acid derivative, followed by unmasking etc. (Scheme 1)



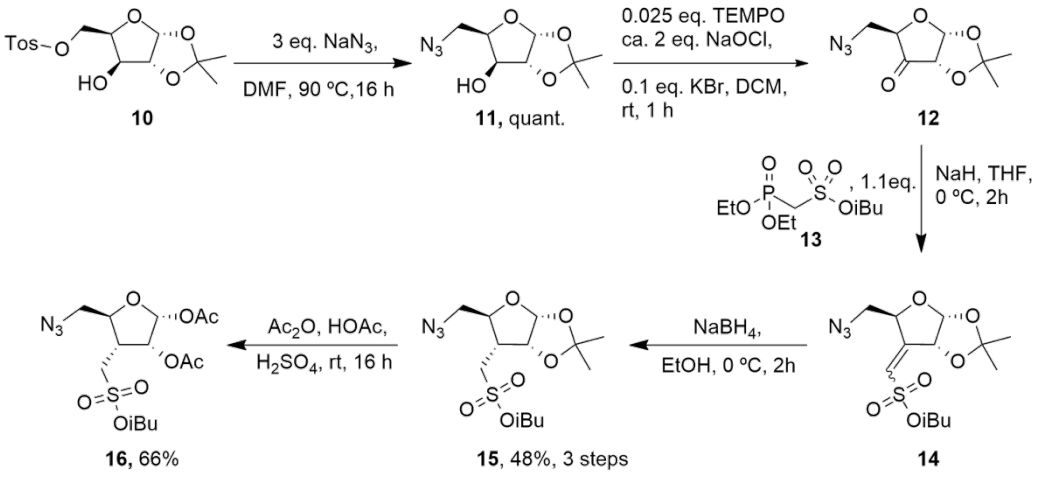
Scheme 1. Required building blocks and the repetitive unmasking and coupling steps for assembly of the sulfonamide backbone

Thus, an easily scalable preparation of the nucleoside synthons was required, which could be converted into activated building blocks in an easy and efficient manner. In the past we have used diacetone-*D*-glucose for preparation of the SaRNA nucleoside synthons.[18] However, it was found that introduction of the required azide onto the 5'-position later in the synthetic route was accompanied by side reactions of the sulfonate group and therefore less efficient (Scheme 2).



Scheme 2. Side reaction of azide with the sulfonate ester

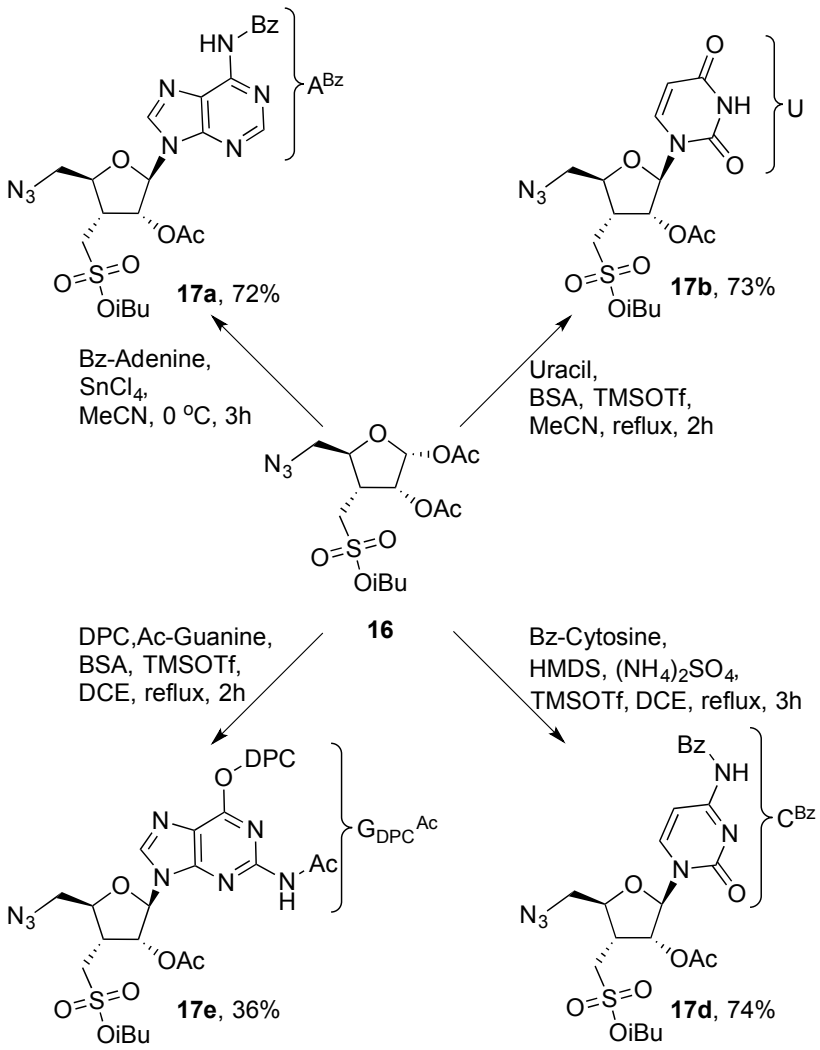
To remedy this a very efficient 5-step synthesis was developed starting from the 5-Tosyl acetone-*D*-xylose **10** which was easily prepared from readily available *D*-xylose, (Scheme 3).[19] In a simple procedure azide **11** was obtained in quantitative yield via a nucleophilic displacement reaction of tosylate **10**.[20] Next, the secondary alcohol functionality of compound **11** was oxidised to ketone **12** in a highly efficient TEMPO radical oxidation.[21] Without further purification the sulfonate ester moiety was introduced via a Horner-Wadsworth-Emmons reaction with phosphonate **13**[22] to give vinyl sulfonate **14** as a mixture of E/Z alkenes which was used without purification.[23] Sodium borohydride reduction of the crude **14** in a Michael reaction was stereoselective because of the steric effect of the acetal moiety and yielded a single isomer of compound **15** in 48% overall yield over 3 steps.[24] The crucial di-acetate synthon **16** to be used for incorporation each of the nucleobases, was obtained in a acetal deprotection/ acetylation step in 66% yield.[25]



Scheme 3. SaRNA nucleoside synthon synthesis

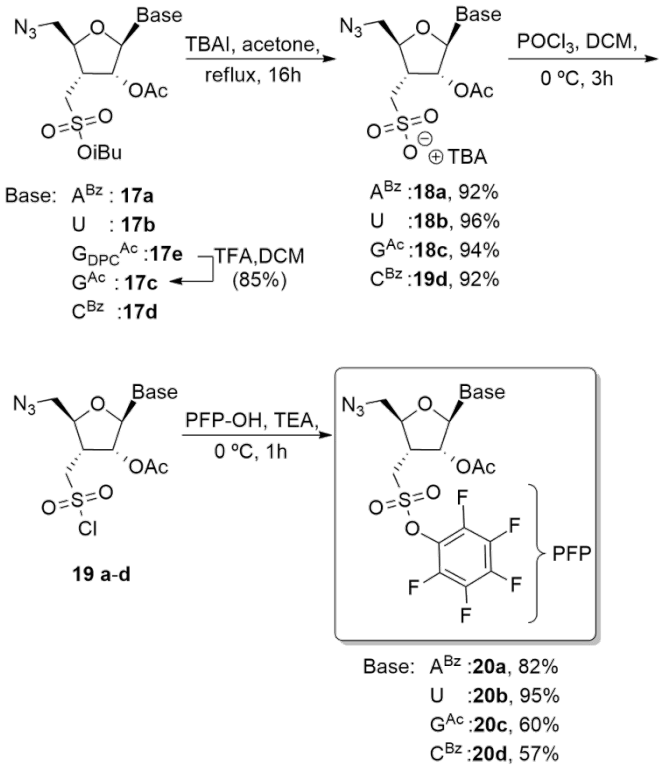
Synthesis of activated SaRNA nucleotide building blocks

Nucleobases were introduced using standard literature procedures and the protected SaRNA nucleotide building blocks **17a,b,e,d** were obtained in acceptable to good yields (44% - 74%, Scheme 4).[26] The nucleobase protecting groups were similar to those used for the synthesis of oligonucleotides analogues with a peptide backbone.[6] Although slight variations in the introduction of the nucleobases is possible, the indicated conditions gave the best yields and reproducible results. For example, it was found that double protection of guanine facilitated purification of the product.



Scheme 4. Synthesis of the S-RNA nucleotide building blocks (BSA: bis-trimethylsilylacetamide, DPC: N,N-Diphenyl Carbamate)

The sulfonamide linkage was introduced using a sulfonamide pentafluorophenyl (PFP) ester instead of the obvious and reactive sulfonyl chloride, since the former is more stable and sufficiently reactive to give rise to formation of sulfonamide linkage in good yields.[27,28] To realize the preparation of SaRNA nucleotide PFP esters **20a-d**, sulfonate esters **17a-d** were converted into sulfonate tetrabutylammonium salts **18a-d** by heating with TBAI.[29] Using tetrabutylammonium sulfonate salts instead of sodium salts afforded compounds that had better solubility properties in organic solvents. This proved to be crucial for the synthesis of sulfonyl chlorides and subsequent PFP esters as the reactions were cleaner and gave rise to higher yields. Also, it was possible to conveniently purify the TBA salts **18a-d** by normal phase flash chromatography. Next, sulfonyl chlorides **19a-d** were obtained using $POCl_3$ in a very clean reaction not requiring any purification for the preparation of the PFP-esters. Other reagents such as $SOCl_2$ in the presence or absence of a catalytic amount of DMF[18] resulted in side reactions with protecting groups on nucleobases that is **19a**, **19c** and **19d**. In a simple nucleophilic substitution reaction required active PFP-esters **20a-d** were obtained in good to excellent yields, excepting **20c**. For synthesis of the latter G-PFP building block **20c**, the DPC-group had to be removed first due to side reactions further through the synthesis (Scheme 5).



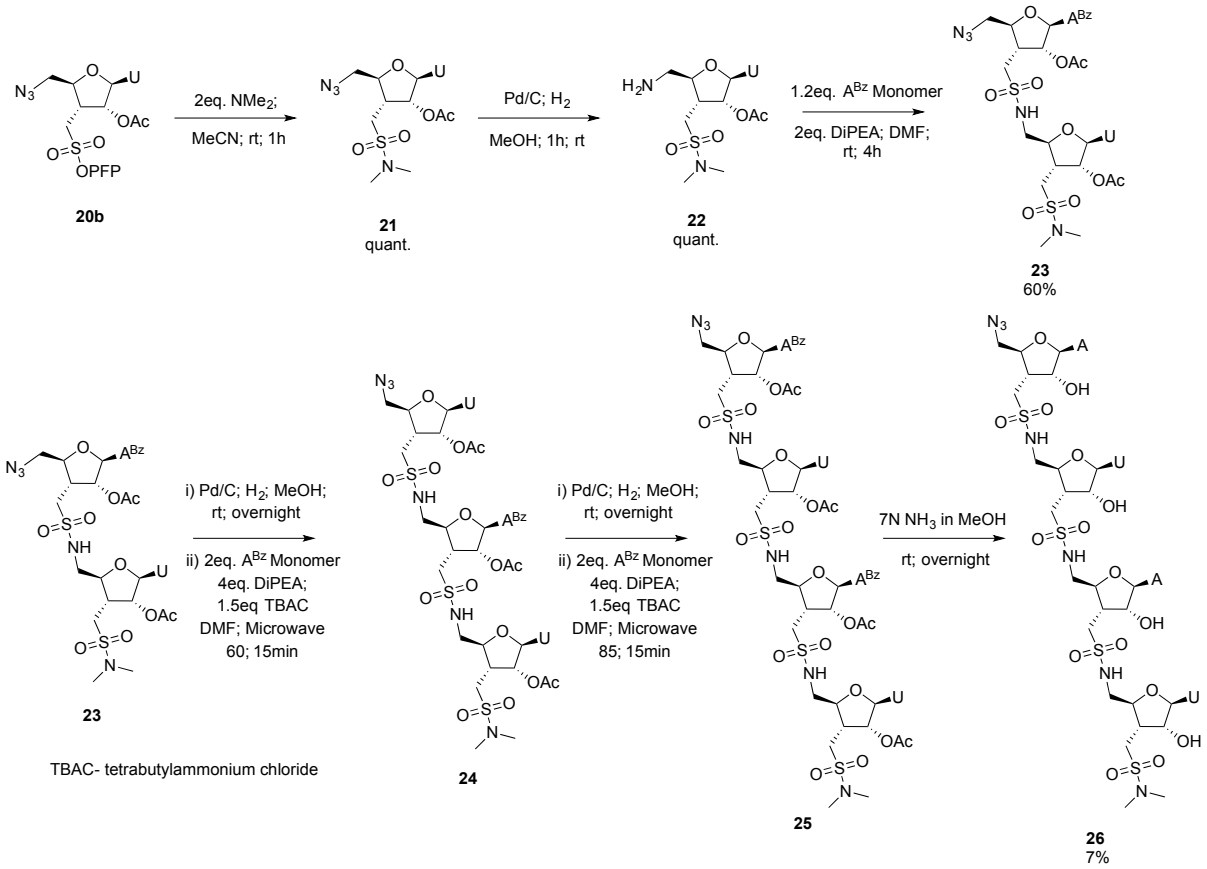
Scheme 5. Synthesis of active (PFP) esters of SaRNA nucleotide building blocks

These nucleotide PFP-esters are the central building blocks to be used for the molecular construction both in solution and in future on solid phase of sulfonamide anti-sense oligonucleotides.

Synthesis in solution of SaRNA

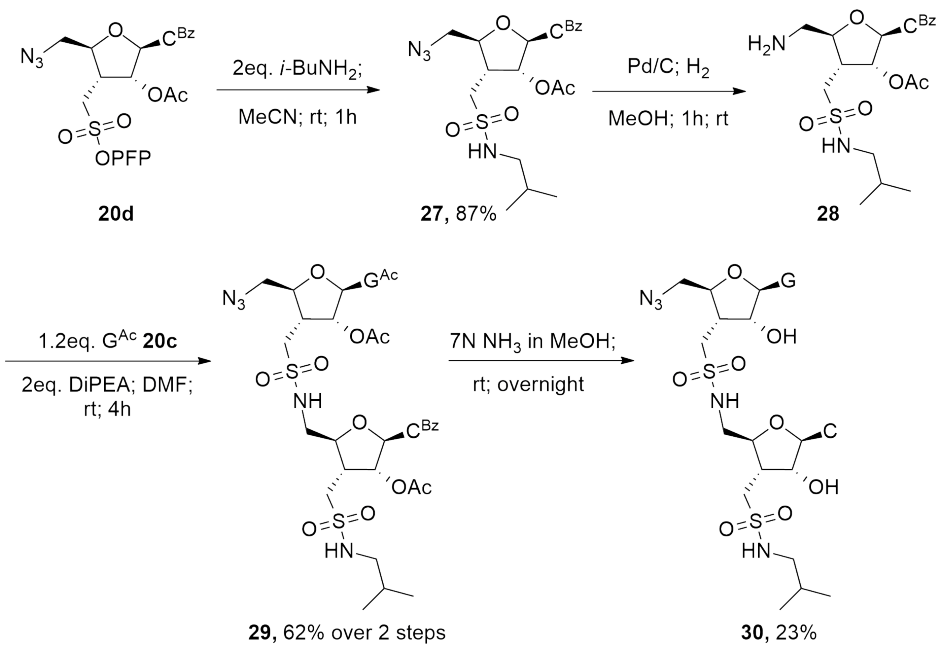
As a first illustration of the use of these PFP-active ester nucleotide building blocks for the synthesis of SaRNA in solution tetranucleotide AUAU (**26**) was selected (Scheme 6). For this the 3'-end nucleotide was obtained by conversion of U-nucleotide building block **20b** to the sulfonamide **21**, of which the azide moiety was unmasked by hydrogenation to give amine **22**.[30] Amine **22** was then coupled to adenine PFP-building block **20a** to give A-U dimer **23**. Dimer **23** was subsequently unmasked as above and immediately coupled to uracil PFP-ester building block **20b**. Since this reaction was slower, coupling was carried out under microwave conditions and tetrabutyl ammonium chloride (TBAC) was added as a nucleophilic catalyst for activation by supposedly *in* situ formation of the sulfonyl chloride as has been described in the literature[27] to give **24**. Repetition of the unmasking and coupling cycle using adenine PFP-building block **20a** again gave the final crude tetranucleotide **25**. After deprotection overnight using a 7M ammonia in methanol solution, the crude SaRNA tetranucleotide **26** was obtained which was purified using preparative HPLC. The resulting SaRNA-tetranucleotide was stable and no decomposition was observed after six months storage at

room temperature.



Scheme 6. Synthesis in solution of SaRNA-tetranucleotide AUAU

To evaluate the removal of the nucleobase-protecting groups in monomers **20c** and **20d**, a GC SaRNA dinucleotide **30** was synthesized in a similar manner as tetranucleotide **26** with efficient removal of the protecting group in an overall yield of 12%, Scheme 7.

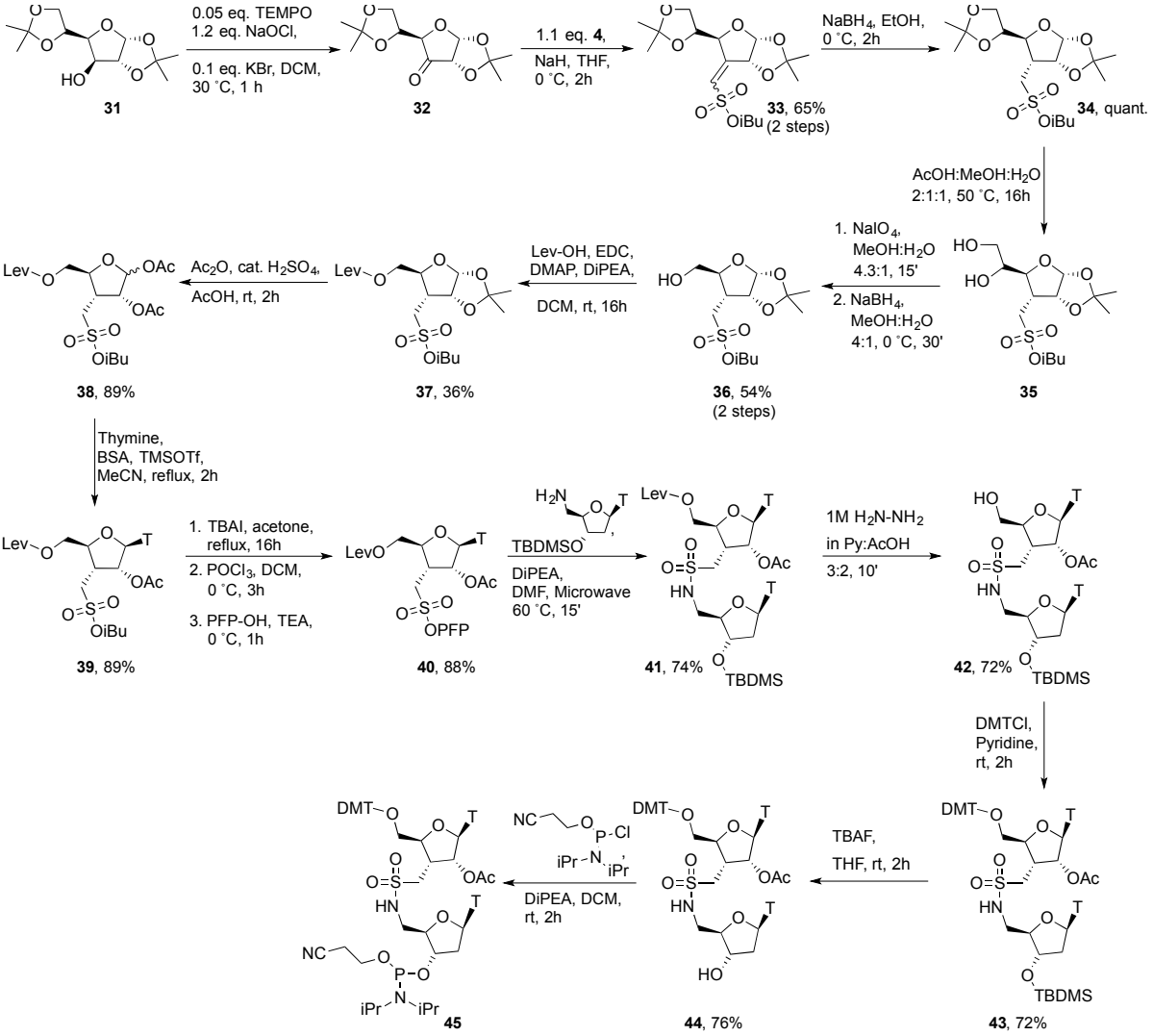


Scheme 7. Synthesis of SaRNA dinucleotide CG

Solid phase synthesis of a DNA-SaRNA hybrid for melting experiments

To assess whether the sulfonamide moiety was indeed a good mimic of the phosphodiester linkage, a single sulfonamide moiety was incorporated into a DNA strand and study the melting behaviour of resulting DNA-SaRNA and compare it to the melting of the corresponding unmodified double-stranded(ds) DNA and ds RNA-DNA. For molecular construction of the required DNA-SaRNA hybrid **46** a sulfonamide containing dinucleotide (**45**) was needed and synthesized (Scheme 8). The resulting dinucleotide had to contain a dimethoxy trityl (DMT) group at the 5' end and a phosphoramidite moiety at the 3' end, so that it would be compatible with standard solid phase synthesis of DNA by the phosphoramidite method. Starting from diacetone-*D*-glucose (**31**) the sulfonate ester moiety was introduced similarly to the preparation of **15** (Scheme 3). In this synthesis di-acetal **34** was converted into diol-acetal **35**, so that one carbon atom could be removed by oxidation,[18] leading ultimately to 5'-levulinic acid protected sulfonate ribose derivative **38**. This derivative was suitable for introduction thymine thus affording **39**. Hydrolysis of the iso-butyl sulfonate ester moiety in **39** could be achieved in the presence of the levulinic ester moiety, after which a 5'-amino 3'-TBDMS protected deoxy-thymidine derivative[31] was reacted with PFP-ester **40** and gave sulfonamide containing dinucleotide derivative **41**. In the final steps the DMT-protective

group was introduced at the 5'-end and the phosphoramidite moiety at the 3'-end, thereby completing the synthesis of sulfonamide containing dinucleotide **45**. This dinucleotide building block was used on a ABI 394 DNA-synthesizer. Thus, the dinucleotide was incorporated in the sequence described by Palframan et al.[32] In this work the influence of replacement of one phosphodiester linkage was conveniently studied.



Scheme 8. Synthesis of a phosphoramidite SaRNA- TT-dinucleotide **45** compatible with standard solid phase DNA-synthesis.

Melting temperatures (T_m) were determined of the parent DNA-DNA* and DNA-RNA hybrids as well as of the **46**-DNA and **46**-RNA hybrids (Figure 3). The incorporation of a sulfonamide linkage led to a decrease in melting temperature, although this decrease was lower, going from a DNA-RNA

hybrid to a **46**-RNA hybrid (Δ T = -4.4°), as compared to going from a DNA-DNA* hybrid to a **46**-RNA hybrid (Δ T = -9.4°). This is important considering possible applications as antisense agents mainly targeting RNA. These results are in agreements with earlier results of Widlanski et al.[13] obtained upon incorporation of sulfonamides of the different geometry with a deoxyribose sugar (Figure 1, SaASO **8**).

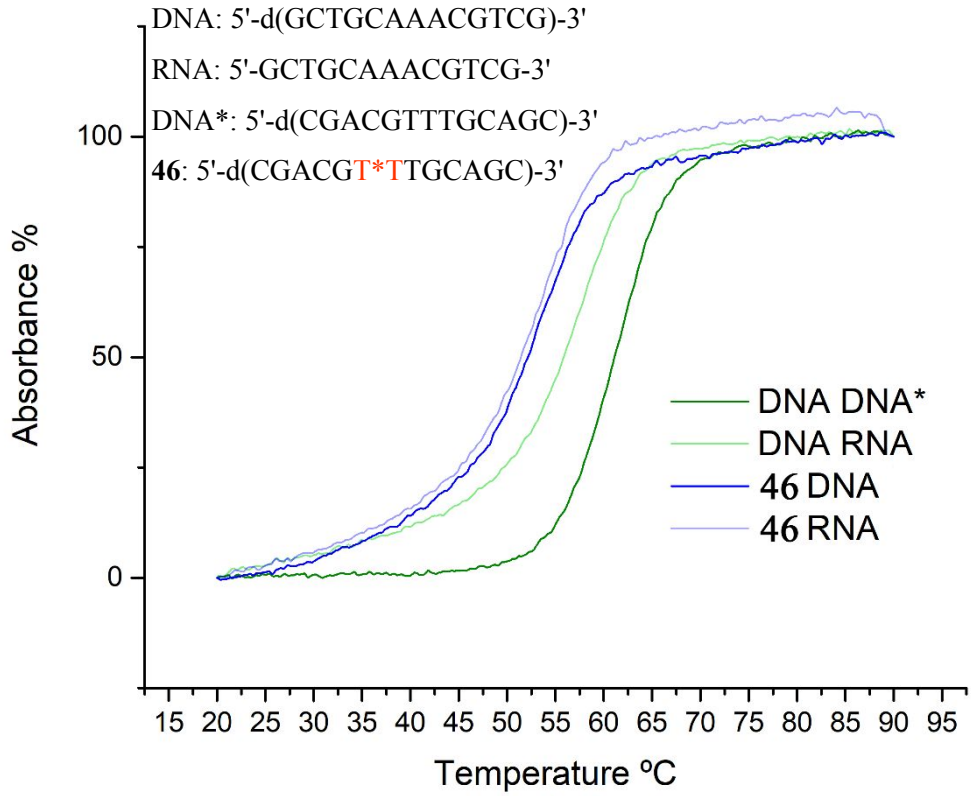


Figure 3. Melting curves of ds SaRNA containing hybrids as compared to those of native ds DNA and ds RNA-DNA

Conclusion

We have developed a versatile synthesis of the complete set of SaRNA nucleotide building blocks with distinct possibilities to expand the synthesis to sulfonamide DNA nucleotide building blocks and other scaffold modifications. Although nucleotide coupling using their PFP esters in solution is optimal, there is room for improvement in light of further development of the solid phase synthesis of sulfonamide based ASOs. In addition, we have developed the synthesis of a sulfonamide containing

dinucleotide building block, which could be used in a standard phosphoramidite solid phase synthesis protocol leading to a DNA-SaRNA hybrid still capable of formation of double-stranded molecules both with DNA and RNA. Although we have the distinct impression that the presented approach for molecular construction of sulfonamide antisense oligonucleotides involving a primary 5'-amine is ultimately better than the approach using the more hindered 3'- amine described in the past by us[18] and others,[17] this still has to be rigorously proven in future research by (solid phase) synthesis of sulfonamide antisense oligonucleotides and/or sulfonamide/phosphodiester chimera of considerable size. Nevertheless, the convenient synthesis of the SaRNA nucleotide building blocks as well as the synthesis of a chimera has demonstrated that molecular construction of sulfonamide ASOs is an achievable goal, with likely applications in ensuing areas, where ASOs are used as therapeutics.

Experimental section

General information

All analytical or HPLC grade chemicals and solvents were purchased from commercial sources and were used as received unless stated otherwise. DNA and RNA sequences were purchased from Eurogentec. Microwave reactions were performed using a CEM Discover® Sp reactor in a sealed glass container with an external IR temperature sensor and magnetic frequency of 2455MHz. Heating of reactions was performed using aluminium heating blocks. ^{1}H NMR, ^{13}C NMR and ^{19}F spectra were recorded on a Bruker AVIII 400 MHz Spectrospin spectrometer in $CDCl_3$. Chemical shifts (δ) are reported in parts per million (ppm) relative to trimethylsilane (TMS, 0.00 ppm), $CDCl_3$ (7.26 ppm) or $CFCl_3$(0.00 ppm). TLC was carried out on silica gel plates (Merck 60F254) and visualization was carried out by both UV detection (254 nm) and staining (anisaldehyde, bromocresol green) followed by heating. For column chromatography, a Biotage®-Isolera™ was used together with Biotage Kp-Sil silica cartridges. Dry solvents (THF, DCM) were dispensed from Pure Solv™ 500 Solvent Purification System and other dry solvents (acetonitrile, DMF) were obtained from freshly opened commercially available HPLC grade solvents by removal of residual water with activated 4 Å

molecular sieves overnight. HRMS-ESI was recorded on Bruker microTOFq High Resolution Mass Spectrometer in the positive or negative mode. Petroleum ether used refers to a mixture with boiling point of 40-60 °C. Liquid chromatography mass spectrometry (LCMS) was carried out on a Thermo Scientific LCQ Fleet quadrupole mass spectrometer with a Dionex Ultimate 3000 LC using a Dr Maisch Reprosil Gold 120 C18 column (110 Å, 3 μm, 150 Å~4.0 mm), using a 0-100% linear gradient of buffer A(95% H_2O, 5% MeCN, 0.1% TFA) into buffer B(95% MeCN, 5% H_2O, 0.1% TFA) in 10 or 40 minutes. Deionised water was obtained on a Milli-Q® station (Merck Millipore). Preparative HPLC was carried out using Angilent Technologies 1260 Infinity Preparative-scale Purification system. Separation was achieved on a Phenomenex Gemini®, 10 μm C18 110˚A AXIA, 250 x 21.2 mm. A linear gradient of 0 → 50% buffer A into buffer B. All runs were conducted over 80 minutes with a flow rate of 12.5 mL/Min. Fraction collection was based on UV absorption detected at both 214 and 254 nm. Analytical HPLC was carried out with a Shimadzu instrument consisting of a communication module (CBM-20A), auto-sampler (SIL-20HT), pump modules(LC-20AT), UV/Vis detector(SPD-20A) and system controller (LabsolutionsV5.54SP), with a Phenomenex Gemini®, 5 μm C18 110˚A AXIA, 250 x4.60 mm. UV measurements were recorded at 214 and 254 nm, by use of a standard protocol using a 0-100% linear gradient of buffer A into buffer B in 40 minutes.

((3aR,5R,6S,6aR)-6-hydroxy-2,2-dimethyltetrahydrofuro[2,3-d][1,3]dioxol-5-yl)methyl 4-methylbenzenesulfonate 10: To a stirring solution of *D*-Xylose (50.0 g, 330 mmol) in acetone (1 L) was added dry $CuSO_4$ (66 g) and conc. H_2SO_4 (5mL). The resulting suspension was stirred at room temperature overnight, after which the reaction mixture was filtered and quenched with conc. NH_4OH (16mL). The newly formed precipitate was filtered off and the supernatant was concentrated *in vacuo* to give a thick syrup, which was dissolved in MeOH : H_2O (4:1, 500 mL) after which 1 M HCl (10 mL) was added. The resulting mixture was stirred for 3 h at room temperature. The volume of the mixture was reduced *in vacuo* and co-evaporated with EtOH and toluene to give a thick syrup. This syrup was dissolved in DCM (300 mL) dried over $MgSO_4$ and concentrated *in vacuo* to give crude protected *D*-xylose derivative (Bozo *et al.*[19]). After dissolving the *D*-Xylose derivative in DCM (900 mL), TEA (93 mL, 670 mmol) was added and the mixture was cooled to 0°C. To the stirring mixture at 0°C was added dropwise a solution of TosCl (69.0 g, 370 mmol) in DCM. The reaction mixture was then warmed to room temperature and stirred overnight. 1 M HCl was added to the reaction mixture and the resulting solution was extracted twice with DCM. The organic layers were washed twice with H_2O, with brine and dried over $MgSO_4$ followed by concentration *in vacuo* to give the crude tosylate **10** which was crystallised from ethanol and gave the desired product as a white solid (71.0 g, 200 mmol, 61%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.81 (d, *J* = 8.3 Hz, 2H), 7.36 (d, *J* = 8.1 Hz, 2H), 5.88 (d, *J* = 3.6 Hz, 1H), 4.51 (d, *J* = 3.6 Hz, 1H), 4.38 – 4.29 (m, 3H), 4.18 – 4.10 (m, 1H), 2.46 (s, 3H), 2.29 (d, *J* = 5.0 Hz, 1H), 1.46 (s, 3H), 1.30 (s, 3H). ^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$) δ 145.3, 132.4, 130.0, 128.0, 112.1, 105.0, 85.0, 77.6, 74.3, 66.1, 26.8, 26.2, 21.7. HRMS (ESI-TOF) m/z: $[M+Na]^+$ Calcd. For $C_{15}H_{20}NaO_7S$ 367.0822 Found 367.0817.

(3aR,5R,6S,6aR)-5-(azidomethyl)-2,2-dimethyltetrahydrofuro[2,3-d][1,3]dioxol-6-ol 11: To a stirring solution of tosylate **10** (71.0 g, 210 mmol) in DMF (500 mL) was added NaN_3 (33.0 g 520 mmol). The resulting reaction mixture was stirred at 90°C overnight. After this, the mixture was concentrated in vacuo to give a thick syrup. EtOAc (500 mL) and water (300 mL) were added to the

resulting concentrate and the aqueous layer was extracted with EtOAc (2 ·). The organic layer was dried over $MgSO_4$ and concentrated in vacuo to give **11** (43.0 g, 210 mmol, quant).

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.96 (d, *J* = 3.7 Hz, 1H), 4.52 (d, *J* = 3.7 Hz, 1H), 4.34 – 4.22 (m, 2H), 3.70 – 3.55 (m, 2H), 2.17 (d, *J* = 4.6 Hz, 1H), 1.51 (s, 3H), 1.32 (s, 3H).^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$) δ 112.0, 104.8, 85.3, 78.2, 75.4, 49.2, 26.8, 26.2. HRMS (ESI-TOF) m/z: $[M+Na]^+$ Calcd For - $C_8H_{13}N_3NaO_4$ 238.0798 Found: 238.0792.

isobutyl ((3aR,5S,6R,6aR)-5-(azidomethyl)-2,2-dimethyltetrahydrofuro[2,3-d][1,3]dioxol-6-yl)methanesulfonate 15: To a vigorously stirring solution of azide **11** (36.0 g, 170 mmol) in DCM (230 mL) was added KBr (2.0 g, 17 mmol) and TEMPO (1.3 g, 8.3 mmol) at room temperature. The resulting mixture was warmed to 30°C and a NaOCl solution (11%-14% active chlorine, 200 mL) was slowly added. The biphasic mixture was stirred for 1 h at 30°C after which ^{1}H NMR of an aliquot of the reaction mixture indicated complete conversion of the starting material. The organic layer was separated and washed with a KI (1.3 g) solution in 0.5 M HCl (100 mL), a saturated $Na_2S_2O_3$ solution (100 mL) and a saturated $NaHCO_3$ (130 mL) solution. The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo* to give crude **12** (36.0g 170mmol) which was used in the next step without further purification. To a stirred cooled (0°C) solution of Horner–Wadsworth–Emmons reagent **13** (9.5 g, 33 mmol) (Carretero *et al.*[22]) in dry THF (250 mL) kept under an N_2 atmosphere, was added NaH (1.6 g of a 60% suspension in mineral oil, 36 mmol). The resulting mixture was stirred for 30 min at 0°C after which a solution of crude ketone **12** (15.0 g, 30 mmol) in THF (50mL) was added dropwise. The reaction mixture was kept at 0°C for 2 h. Water (50 mL) was then added to quench the reaction mixture and it was warmed to room temperature. THF was removed *in vacuo* and the resulting aqueous mixture was diluted with water and extracted with DCM. The combined organic layers were dried over $MgSO_4$, filtered and concentrated *in vacuo* to give crude sulfonate **14** which was dissolved in EtOH (600 mL). To the resulting stirred solution was added $NaBH_4$ (6.8 g, 180 mmol) in portions over a period of 10 min at 0°C. The resulting mixture was stirred for 2 h at 0°C. AcOH (10mL) was then added to quench the reaction mixture. The resulting mixture was stirred for an additional 15 min at room temperature and the solvent was removed *in vacuo*. The

obtained solid was partitioned between an aqueous saturated $NaHCO_3$ solution and EtOAc. The EtOAc layer was washed with brine dried over $MgSO_4$ filtered and concentrated *in vacuo* to give crude reduced sulfonate **15** which was purified using flash chromatography on silica gel using a gradient from 0% to 20% EtOAc in hexanes and gave the desired product as a white solid (6.7 g, 19 mmol, 48% over three steps).

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.87 (d, *J* = 3.6 Hz, 1H), 4.84 (t, *J* = 4.1 Hz, 1H), 4.03 (dd, *J* = 6.5, 0.7 Hz, 2H), 3.99 (dt, *J* = 10.0, 4.0 Hz, 1H), 3.66 (dd, *J* = 13.4, 3.7 Hz, 1H), 3.56 (dd, *J* = 14.6, 9.6 Hz, 1H), 3.41 (dd, *J* = 13.5, 4.2 Hz, 1H), 3.09 (dd, *J* = 14.6, 3.5 Hz, 1H), 2.59 (tdd, *J* = 9.8, 4.6, 3.6 Hz, 1H), 2.12 – 1.97 (m, 1H), 1.52 (s, 3H), 1.35 (s, 3H), 1.01 (d, *J* = 1.6 Hz, 3H), 0.99 (d, *J* = 1.6 Hz, 3H). ^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$) δ 112.3, 104.8, 80.4, 78.7, 76.1, 51.3, 45.8, 41.1, 28.3, 26.7, 26.4, 18.7, 18.6, 14.2. HRMS (ESI-TOF) m/z: $[M+Na]^+$ Calcd. for - $C_{13}H_{23}N_3NaO_6S$ 372.1200 Found 372.1184.

(2R,3R,4R,5S)-5-(azidomethyl)-4-((isobutoxysulfonyl)methyl)tetrahydrofuran-2,3-diyl diacetate 16: To a solution of **15** (3.6 g, 10 mmol) in AcOH (60 mL), acetic anhydride (9.1 mL) and a catalytic amount (drop) of conc. H_2SO_4 were added at 0°C. The resulting mixture was stirred for 6 h at room temperature. The reaction mixture was then diluted with DCM (200mL) and slowly poured into an ice cold sat. $NaHCO_3$ solution (500 mL). The organic layer was washed with brine, dried over $MgSO_4$, filtered and concentrated *in vacuo* to give crude **16**, which was purified using flash chromatography on silica gel using a gradient from 0% to 20% EtOAc in hexanes and gave the desired product as a colourless oil, yield (2.6 g, 6.6 mmol, 66%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 6.13 (s, 1H), 5.33 (d, *J* = 4.8 Hz, 1H), 4.21 (dt, *J* = 9.2, 4.0 Hz, 1H), 4.07 – 3.99 (m, 2H), 3.68 (dd, *J* = 13.4, 4.0 Hz, 1H), 3.50 – 3.34 (m, 2H), 3.16 (dd, *J* = 14.3, 5.3 Hz, 1H), 3.14 – 3.06 (m, 1H), 2.14 (s, 3H), 2.12 (s, 5H), 2.10 – 2.01 (m, 1H), 1.01 (s, 4H), 0.99 (s, 3H). ^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$) δ 169.4, 169.0, 98.2, 82.4, 76.0, 52.5, 46.4, 37.2, 28.3, 21.1, 20.6, 18.6; HRMS (ESI-TOF) m/z: $[M+Na]^+$ Calcd. For - $C_{14}H_{23}N_3NaO_8S$ 416.1098 Found 416.1090.

(2R,3R,4R,5S)-5-(azidomethyl)-2-(6-benzamido-9H-purin-9-yl)-4-((isobutoxysulfonyl)methyl)tetrahydrofuran-3-yl acetate 17a: To a stirred solution of **16** (1.7g, 4.3 mmol) in MeCN (20 mL) was added 6-*N*-benzoyladenine (2.2 g, 9.0 mmol) and $SnCl_4$ (2.0 mL, 17 mmol) at 0°C. The resulting suspension was stirred for 3h at 0 ºC during which the reaction mixture became clear. Next, the reaction mixture was diluted with 50 mL of DCM and quenched with an aqueous saturated $NaHCO_3$ solution (50mL) at 0°C. The organic layer was separated and the aqueous layer was extracted twice with DCM (2 · 100 mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated *in vacuo* to give crude **17a** which was purified using flash chromatography on silica gel using a gradient of 0% to 10% MeOH in DCM. The desired product was obtained as a white foam (1.7 g, 3.1 mmol, 72%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 9.00 (s, 1H), 8.82 (s, 1H), 8.17 (s, 1H), 8.08 – 8.02 (m, 2H), 7.68 – 7.61 (m, 1H), 7.59 – 7.52 (m, 2H), 6.07 (d, *J* = 1.0 Hz, 1H), 5.89 (d, *J* = 5.9 Hz, 1H), 4.36 – 4.27 (m, 1H), 4.10 (dd, *J* = 6.5, 2.4 Hz, 2H), 4.00 – 3.91 (m, 1H), 3.82 (dd, *J* = 13.3, 3.8 Hz, 1H), 3.73 (dd, *J* = 13.4, 4.8 Hz, 1H), 3.56 (dd, *J* = 14.6, 8.9 Hz, 1H), 3.33 (dd, *J* = 14.6, 5.0 Hz, 1H), 2.23 (s, 3H), 2.15 – 2.04 (m, 1H), 1.05 (s, 3H), 1.03 (s, 3H); ^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$) δ 169.8, 142.1, 132.9, 128.9, 127.8, 90.0, 82.4, 77.8, 76.1, 52.0, 46.3, 38.5, 28.4, 20.7, 18.6. HRMS (ESI-TOF) m/z: $[M+Na]^+$ Calcd. for – $C_{16}H_{23}N_5NaO_8S$ 468.1160 Found 468.1168.

(2R,3R,4R,5S)-5-(azidomethyl)-2-(2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-4-((isobutoxysulfonyl)methyl)tetrahydrofuran-3-yl acetate 17b: To a stirred solution of **16** (135mg, 0.34 mmol) in dry MeCN (5 mL) under a nitrogen atmosphere was added uracil (58 mg, 0.51 mmol) and N,O-bis(trimethylsilyl)acetamide (290 µL, 1.2 mmol) at room temperature. The resulting mixture was stirred at reflux for 1h and then cooled down to room temperature. TMSOTf (120 µL, 0.69 mmol) was added and the resulting mixture was stirred at reflux for 1h. A sat. $NaHCO_3$ solution (30 mL) was added to quench the reaction mixture followed by addition of DCM (30mL). The aqueous layer was extracted with DCM (2 · 30 mL) and the combined organic layers were dried over $MgSO_4$, filtered and concentrated in vacuo to give crude **17b** which was purified using flash chromatography

on silica gel using a 20% to 80% gradient of EtOAc in hexanes and the desired product was obtained as a white solid (110 mg, 0.25 mmol, 73%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.14 (s, 1H), 7.17 (d, *J* = 1.2 Hz, 1H), 5.62 (d, *J* = 2.7 Hz, 1H), 5.54 (dd, *J* = 7.1, 2.7 Hz, 1H), 4.18 (dt, *J* = 8.7, 3.7 Hz, 1H), 4.07 (dd, *J* = 6.6, 1.0 Hz, 2H), 3.83 (dd, *J* = 13.5, 3.2 Hz, 1H), 3.67 (dd, *J* = 13.5, 4.1 Hz, 1H), 3.47 (dd, *J* = 14.3, 7.6 Hz, 1H), 3.35 – 3.27 (m, 1H), 3.18 (dd, *J* = 14.3, 6.1 Hz, 1H), 2.19 (s, 3H), 2.12 – 2.04 (m, 1H), 1.03 (s, 3H), 1.02 (s, 3H).^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$) δ 169.6, 162.5, 149.7, 140.9, 103.2, 92.2, 81.4, 76.6, 76.2, 51.9, 46.7, 37.7, 28.4, 20.6, 18.6. HRMS (ESI-TOF) m/z: $[M+Na]^+$ Calcd. for – $C_{24}H_{28}N_8NaO_7S$ 595.1680 Found 595.1694.

(2R,3R,4R,5S)-2-(2-acetamido-6-((diphenylcarbamoyl)oxy)-9H-purin-9-yl)-5-(azidomethyl)-4-((isobutoxysulfonyl)methyl)tetrahydrofuran-3-yl acetate 17e: To a stirring suspension of 2-N-acetyl-6-O-diphenylcarbamoylguanine (990 mg 2.54 mmol) in DCE (7 mL) was added N,O-Bis(trimethyl)acetamide (1.20 mL, 5.08 mmol). The resulting suspension was refluxed for 10 min and then cooled down to room temperature. A solution of compound **16** (500 mg**,** 1.24 mmol) in DCE (3 mL) was added to the reaction mixture. TMSOTf (0.43 mL, 2.5 mmol) was then added dropwise at 0°C, and thereafter the reaction mixture was refluxed for 1h, the reaction was quenched at 0°C with saturated aqueous $NaHCO_3$ (5 mL), diluted in DCM (20 mL) and the organic layer was separated. The aqueous layer was extracted with DCM (3 · 20 mL) and the combined organic layers were washed with sat. $NaHCO_3$ (20 mL) and brine (3 · 20 mL), dried over $MgSO_4$ and concentrated *in vacuo*. The crude, obtained as a yellow foam, was purified by column chromatography using a gradient of 0% to 5% of MeOH in DCM to give the desired product **17e** as a white foam (330 mg, 0.46mmol, 36%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.12 (s, 1H), 8.02 (s, 1H), 7.44 – 7.35 (m, 8H), 5.90 (s, 1H), 5.81 (d, *J* = 5.3 Hz, 1H), 4.22 (m, 1H), 4.04 (tt, *J* = 9.4, 4.8 Hz, 2H), 3.76 (t, *J* = 4.4 Hz, 2H), 3.52 (dd, *J* = 14.4, 9.6 Hz, 1H), 3.33 (dd, *J* = 14.4, 4.3 Hz, 1H), 2.39 (s, 3H), 2.13 (d, *J* = 9.7 Hz, 1H), 0.97 (d, *J* = 7.0, 6H). ^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$) δ 171.1, 169.8, 156.5, 153.8, 151.9, 150.2, 143.1, 121.5, 90.0, 82.3, 77.7, 76.1, 60.4, 52.1, 46.2, 38.4, 28.3, 25.1, 21.0, 20.6, 18.6, 18.6, 14.2. HRMS (ESI-TOF) m/z: $[M+Na]^+$ Calcd. for – $C_{32}H_{35}N_9NaO_9S$ 744.2150 Found 744.2171.

(2R,3R,4R,5S)-5-(azidomethyl)-2-(4-benzamido-2-oxopyrimidin-1(2H)-yl)-4-((isobutoxysulfonyl)methyl)tetrahydrofuran-3-yl acetate 17d: To a stirring suspension of N4-Benzoylcytosine (500 mg, 2.30 mmol) in (5 mL) was added $(NH_4)_2SO_4$ (2.1 mg). This suspension was refluxed for 3 h, until a clear solution was obtained. The mixture was concentrated and co-evaporated three times with toluene, and a solution of **16** (400 mg, 1.01 mmol) in DCE (4 mL) was added to the residue. TMSOTf (0.36 mL, 2.02 mmol) was then added dropwise at 0°C, and the reaction mixture was refluxed for 1h. The reaction was quenched at 0°C with saturated aqueous $NaHCO_3$ (5 mL), diluted with DCM (20 mL) and organic layer was separated. The aqueous layer was extracted with DCM (3 · 20 mL) and the combined organic layers were washed with saturated aqueous $NaHCO_3$ (60 mL) and brine (3 · 60 mL), dried over $MgSO_4$ and concentrated *in vacuo*. The crude product, obtained as a yellow foam, was purified by silica gel column chromatography using a gradient 0% to 5% MeOH in DCM to give the desired product **17d** as a white foam (410 mg, 0.74 mmol, 74%).

^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.69 (s, 1H), 7.98 – 7.87 (m, 3H), 7.66 – 7.59 (m, 1H), 7.55 – 7.48 (m, 2H), 5.72 (d, *J* = 2.1 Hz, 1H), 5.65 (dd, *J* = 6.6, 2.1 Hz, 1H), 4.26 (dt, *J* = 9.0, 3.7 Hz, 2H), 4.04 (dd, *J* = 6.5, 1.4 Hz, 2H), 3.89 (dd, *J* = 13.4, 3.2 Hz, 1H), 3.81 (dd, *J* = 13.5, 4.3 Hz, 1H), 3.48 (dd, *J* = 14.4, 6.6 Hz, 1H), 3.39 – 3.26 (m, 1H), 3.18 (dd, *J* = 14.4, 6.7 Hz, 1H), 2.17 (s, 3H), 2.12 – 1.99 (m, 1H), 1.00 (s, 3H), 0.99 (s, 3H). ^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$) δ 169.6, 133.3, 129.1, 93.5, 82.3, 76.4, 52.0, 46.9, 37.7, 28.3, 20.7, 18.6. HRMS (ESI-TOF) m/z: $[M+Na]^+$ Calcd. for – $C_{23}H_{28}N_6NaO_8S$ 571.1582 Found 571.1581.

(2R,3R,4R,5S)-2-(2-acetamido-6-oxo-1H-purin-9(6H)-yl)-5-(azidomethyl)-4-((isobutoxysulfonyl)methyl)tetrahydrofuran-3-yl acetate 17c: Compound **17e** (330 mg 0.45 mmol) was dissolved in a solution of 1:1 DCM:TFA (5 mL) and stirred at room temperature for 1 h after which it was concentrated *in vacuo*. The residue was dissolved in DCM, washed with an aqueous saturated $NaHCO_3$ (20 mL) solution, brine (3 · 20 mL), dried over $MgSO_4$ and concentrated *in vacuo*. The crude product was obtained as a yellow foam and purified by column chromatography using a

gradient of 0% to 5% MeOH in DCM to give the desired product **17c** as a white foam (200 mg, 0.38 mmol, 85%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 11.84 (s, 1H), 9.04 (s, 1H), 7.73 (s, 1H), 6.13 (d, *J* = 4.7 Hz, 1H), 5.80 (s, 1H), 4.39 (s, 1H), 4.21 (dt, *J* = 10.6, 4.1 Hz, 1H), 4.11 – 4.00 (m, 2H), 3.64 (dd, *J* = 13.5, 4.1 Hz, 1H), 3.52 (dd, *J* = 14.1, 10.6 Hz, 1H), 3.42 (dd, *J* = 13.5, 4.0 Hz, 1H), 3.26 (dd, *J* = 14.1, 3.0 Hz, 1H), 2.25 (s, 3H), 2.19 (s, 3H), 2.11 – 1.99 (m, 1H), 1.00 (s, 3H), 0.99 (s, 3H). ^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$) δ 171.6, 169.7, 155.3, 147.1, 146.8, 139.2, 121.8, 89.5, 81.3, 76.5, 51.5, 45.6, 38.3, 28.3, 24.2, 20.7, 18.6. HRMS (ESI-TOF) m/z: $[M+Na]^+$ Calcd. for - $C_{32}H_{35}N_9NaO_9S$ 744.2150; Found 744.2171.

tetrabutylammonium ((2S,3R,4R,5R)-4-acetoxy-2-(azidomethyl)-5-(6-benzamido-9H-purin-9-yl)tetrahydrofuran-3-yl)methanesulfonate 18a: To a stirred solution of **17a** (110 mg, 0.53 mmol) in 10 mL of acetone was added TBAI (300 mg, 0.80 mmol). The resulting solution was stirred at reflux for 22 h after which LC/MS analysis showed completion of the reaction. Next, the solvent was removed *in vacuo* and the crude mixture was purified using a gradient of 0% to 15% MeOH in DCM affording the desired product **18a** as a white foam (370 mg, 0.49 mmol, 92%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 9.15 (s, 1H), 8.81 (s, 1H), 8.43 (s, 1H), 8.01 (d, *J* = 7.6 Hz, 2H), 7.59 (d, *J* = 7.3 Hz, 1H), 7.51 (t, *J* = 7.6 Hz, 2H), 6.18 (d, *J* = 1.5 Hz, 1H), 5.85 (d, *J* = 5.9 Hz, 1H), 4.53 – 4.40 (m, 1H), 4.08 – 3.84 (m, 1H), 3.36 (s, 1H), 3.32 – 3.22 (m, 8H), 3.17 (dd, *J* = 13.8, 6.0 Hz, 1H), 2.95 (dd, *J* = 13.8, 7.8 Hz, 1H), 2.13 (s, 3H), 1.70 – 1.57 (m, 8H), 1.53 – 1.34 (m, 8H), 0.98 (t, *J* = 7.3 Hz, 12H). ^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$) δ 169.9, 164.6, 152.8, 151.4, 149.4, 141.6, 133.7, 132.7, 128.8, 127.8, 123.1, 88.4, 83.9, 78.7, 58.8, 52.8, 47.5, 39.4, 24.0, 20.8, 19.7, 13.6. HRMS (ESI-TOF) m/z: $[M-H]^-$ Calcd. for - $C_{20}H_{19}N_8O_7S$ 515.1080; Found 515.1103.

tetrabutylammonium ((2S,3R,4R,5R)-4-acetoxy-2-(azidomethyl)-5-(2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)tetrahydrofuran-3-yl)methanesulfonate 18b: Compound **17b** was synthesized according to the procedure used for the preparation of **18a**. Purification by flash chromatography on silica gel using a 0% to 15% gradient of MeOH in DCM gave the desired product **9a** as a white foam (150 mg, 0.24 mmol, 96%).

^{1}H NMR (400 MHz, , $CDCl_3$) δ 8.81 (s, 1H), 7.64 (d, *J* = 8.1 Hz, 1H), 5.88 (d, *J* = 2.5 Hz, 1H), 5.73 (d, *J* = 8.1 Hz, 1H), 5.47 (dd, *J* = 6.9, 2.6 Hz, 1H), 4.38 – 4.30 (m, 1H), 3.97 (dd, *J* = 13.3, 2.7 Hz, 1H), 3.91 (dd, *J* = 13.4, 4.2 Hz, 1H), 3.36 – 3.22 (m, 8H), 3.10 (dd, *J* = 13.6, 5.1 Hz, 1H), 3.06 – 2.96 (m, 1H), 2.84 (dd, *J* = 13.6, 8.0 Hz, 1H), 2.09 (s, 3H), 1.72 – 1.59 (m, 8H), 1.53 – 1.39 (m, 8H), 1.01 (t, *J* = 7.3 Hz, 12H). ^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$) δ 169.5, 162.9, 149.9, 140.1, 102.7, 89.1, 82.8, 77.6, 58.8, 52.7, 47.6, 39.1, 24.0, 20.7, 19.7, 13.7. HRMS (ESI-TOF) m/z: $[M-H]^-$ Calcd. for - $C_{12}H_{14}N_5O_8S$ 388.0569; Found 388.0564.

tetrabutylammonium ((2S,3R,4R,5R)-4-acetoxy-2-(azidomethyl)-5-(4-benzamido-2-oxopyrimidin-1(2H)-yl)tetrahydrofuran-3-yl)methanesulfonate 18c: Compound **18c** was synthesized according to the procedure used for the preparation of **18a**. Purification by flash chromatography on silica gel using a gradient from 0% to 15% MeOH in DCM gave the desired product **18c** as a white foam (230 mg, 0.32 mmol, 94%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 11.81 (s, 1H), 11.35 (s, 1H), 7.62 (s, 1H), 6.36 (d, *J* = 4.4 Hz, 1H), 5.76 (s, 1H), 4.67 – 4.57 (m, 1H), 4.19 (ddd, *J* = 11.2, 6.3, 2.5 Hz, 1H), 3.64 (dd, *J* = 13.5, 2.6 Hz, 1H), 3.40 – 3.15 (m, 8H), 2.84 (dd, *J* = 13.7, 2.9 Hz, 1H), 2.27 (s, 3H), 2.14 (s, 3H), 1.74 – 1.60 (m, 8H), 1.52 – 1.36 (m, 8H), 0.99 (t, *J* = 7.3 Hz, 12H). ^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$) δ 173.2, 170.5, 156.0, 147.7, 147.4, 139.2, 121.2, 89.7, 82.9, 78.3, 58.9, 52.8, 45.7, 40.0, 24.2, 23.9, 20.9, 19.7, 13.6. HRMS (ESI-TOF) m/z: $[M-H]^-$ Calcd. for - $C_{15}H_{17}N_8O_8S$ 469.0896; Found 469.0891.

tetrabutylammonium ((2S,3R,4R,5R)-4-acetoxy-2-(azidomethyl)-5-(4-benzamido-2-oxopyrimidin-1(2H)-yl)tetrahydrofuran-3-yl)methanesulfonate 18d: Compound **18d** was synthesized according to the procedure used for the preparation of **18a**. Purification by flash chromatography on silica gel using a gradient from 0% to 15% MeOH in DCM gave the desired product **18d** as a white foam (260 mg, 0.36 mmol, 92%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.67 (s, 1H), 8.27 (s, 1H), 7.92 (d, *J* = 7.6 Hz, 2H), 7.68 – 7.43 (m, 3H), 5.97 (s, 1H), 5.60 (d, *J* = 5.6 Hz, 1H), 4.50 – 4.38 (m, 1H), 4.22 – 3.99 (m, 2H), 3.39 – 3.27 (m, 8H), 3.10 (dd, *J* = 13.7, 3.9 Hz, 1H), 3.04 – 2.92 (m, 1H), 2.83 (dd, *J* = 13.7, 9.0 Hz, 1H), 2.17 (s,

2H), 2.10 (s, 3H), 1.74 – 1.59 (m, 8H), 1.56 – 1.37 (m, 8H), 1.01 (t, J = 7.3 Hz, 12H). $^{13}C\{^{1}H\}$ NMR (101 MHz, Chloroform-*d*) δ 169.2, 133.1, 129.0, 83.7, 78.3, 58.9, 52.7, 47.9, 38.8, 30.9, 24.1, 20.8, 19.8, 13.7. HRMS (ESI-TOF) m/z: $[M-H]^-$ Calcd. for - $C_{19}H_{19}N_6O_8S$ 491.0991; Found 491.0969.

(2R,3R,4R,5S)-5-(azidomethyl)-2-(6-benzamido-9H-purin-9-yl)-4-(((perfluorophenoxy)sulfonyl)methyl)tetrahydrofuran-3-yl acetate 20a: To a stirred solution of compound **18a** (760 mg, 1.00 mmol) in acetonitrile (10 mL) was added $POCl_3$ (470 µL, 5.00 mmol) at 0°C. The resulting mixture was stirred at room temperature for 6h. The mixture was then concentrated in vacuo to give crude **19a** as a foam, which was dissolved in dry DCM (10 mL) under a N_2 atmosphere. To this stirred, cooled (0°C) solution were added PFPOH (740 mg, 4.00 mmol) and NMM (440 µL, 4.00 mmol). Then, the mixture was warmed to room temperature and stirred for an additional 1h. The reaction mixture was diluted with DCM (40 mL) and quenched with 1 M HCl (10 mL). The organic layer was separated and the aqueous layer was extracted twice with DCM. The organic layers were washed with brine, dried over $MgSO_4$, filtered and concentrated *in vacuo* to give crude **20a** which was purified by flash chromatography on silica gel using a gradient from 0% to 10% MeOH in DCM affording the desired product as a white foam (560 mg, 0.82 mmol, 82%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.92 (s, 1H), 8.81 (s, 1H), 8.14 (s, 1H), 8.03 (d, J = 7.7 Hz, 2H), 7.62 (t, J = 7.2 Hz, 1H), 7.54 (t, J = 7.7 Hz, 2H), 6.06 (s, 1H), 5.92 (d, J = 5.9 Hz, 1H), 4.33 (dt, J = 9.2, 4.5 Hz, 1H), 4.24 – 4.15 (m, 1H), 3.92 (dd, J = 14.6, 9.4 Hz, 1H), 3.83 (dd, J = 13.3, 4.4 Hz, 1H), 3.78 (d, J = 4.8 Hz, 1H), 3.73 (dd, J = 14.3, 4.4 Hz, 1H), 2.22 (s, 3H). $^{13}C\{^{1}H\}$ NMR (101 MHz, $CDCl_3$) δ 169.7, 164.6, 152.8, 151.1, 149.9, 142.1, 133.5, 132.9, 128.9, 127.9, 123.6, 90.1, 82.0, 77.6, 53.5, 51.9, 48.6, 38.7, 20.5. ^{19}F NMR (376 MHz, $CDCl_3$) δ -150.85 – -151.65 (m), -153.65 – -154.50 (m), -159.64 – -160.59 (m). HRMS (ESI-TOF) m/z: $[M+Na]^+$ Calcd. for - $C_{26}H_{19}F_5N_8NaO_7S$ 705.0916; Found 705.0906.

(2R,3R,4R,5S)-5-(azidomethyl)-2-(2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-4-(((perfluorophenoxy)sulfonyl)methyl)tetrahydrofuran-3-yl acetate 20b: Compound **20b** was synthesized according to the procedure used for the preparation of **20a**. Purification by flash

chromatography on silica gel using a gradient from 0% to 10% of MeOH in DCM gave the desired product **11b** as a white foam yield (95 mg, 0.17 mmol, 95%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32 (d, *J* = 8.1 Hz, 1H), 5.81 (dd, *J* = 8.1, 1.8 Hz, 1H), 5.60 (dd, *J* = 6.8, 2.3 Hz, 1H), 5.56 (d, *J* = 2.3 Hz, 1H), 4.20 (dt, *J* = 8.3, 4.1 Hz, 1H), 3.90 – 3.80 (m, 2H), 3.71 (dd, *J* = 13.4, 4.4 Hz, 1H), 3.65 – 3.51 (m, 2H), 2.19 (s, 3H). ^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$) δ 169.7, 149.8, 141.5, 103.2, 93.5, 81.2, 76.5, 51.9, 49.1, 38.1, 20.5. ^{19}F NMR (376 MHz, $CDCl_3$) δ -150.75 – -151.58 (m), -159.71 – -160.44 (m), -159.72 – -160.65 (m). HRMS (ESI-TOF) m/z: $[M+Na]^+$ Calcd. for - $C_{18}H_{14}F_5N_5NaO_8S$ 578.0381; Found 578.0379.

(2R,3R,4R,5S)-2-(2-acetamido-6-oxo-1H-purin-9(6H)-yl)-5-(azidomethyl)-4-(((perfluorophenoxy)sulfonyl)methyl)tetrahydrofuran-3-yl acetate 20c: Compound **20c** was synthesized according to the procedure used for the preparation of **20a.** Purification by flash chromatography on silica gel using a gradient from 0% to 15% MeOH in DCM gave the desired product **20c** as a white foam (120 mg, 0.19 mmol, 60%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 11.86 (s, 1H), 8.67 (s, 1H), 7.69 (s, 1H), 6.20 (d, *J* = 4.8 Hz, 1H), 5.82 (s, 1H), 4.46 (t, *J* = 10.5 Hz, 0H), 4.27 (dt, *J* = 9.9, 4.4 Hz, 1H), 3.92 (dd, *J* = 14.4, 10.6 Hz, 1H), 3.76 – 3.64 (m, 2H), 3.51 (dd, *J* = 13.5, 3.9 Hz, 1H), 2.25 (s, 3H), 2.20 (s, 3H). ^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$) δ 171.4, 169.6, 155.3, 147.1, 146.8, 139.0, 122.2, 89.6, 80.8, 76.2, 51.5, 48.2, 38.7, 24.2, 20.5. ^{19}F NMR (376 MHz, $CDCl_3$) δ -151.78 – -151.93 (m), -153.14 – -153.40 (m), -159.55 – -159.84 (m). HRMS (ESI-TOF) m/z: $[M+Na]^+$ Calcd. for – $C_{21}H_{17}F_5N_8NaO_8S$ 659.0702; Found 659.0702.

(2R,3R,4R,5S)-5-(azidomethyl)-2-(4-benzamido-2-oxopyrimidin-1(2H)-yl)-4-(((perfluorophenoxy)sulfonyl)methyl)tetrahydrofuran-3-yl acetate 20d: Compound **20d** was synthesized according to the procedure used for the preparation of **20a**. Purification by flash chromatography on silica gel using a gradient from 0% to 10% of MeOH in DCM gave the desired product **20d** as a white foam (190 mg, 0.29 mmol, 57%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.90 (d, *J* = 7.5 Hz, 2H), 7.86 (d, *J* = 7.5 Hz, 1H), 7.66 – 7.58 (m, 2H), 7.52 (t, *J* = 7.6 Hz, 2H), 5.72 (dd, *J* = 6.3, 2.0 Hz, 1H), 5.68 (d, *J* = 2.0 Hz, 1H), 4.28 (dd, J = 8.3, 3.9

Hz, 1H), 3.94 – 3.77 (m, 3H), 3.66 – 3.54 (m, 2H), 2.18 (s, 1H); $^{13}C\{^{1}H\}$ NMR (101 MHz, $CDCl_3$) δ 169.6, 133.3, 129.1, 127.6, 94.6, 82.0, 77.1, 52.1, 49.4, 38.1, 20.6; ^{19}F NMR (376 MHz, $CDCl_3$) δ -150.91 – -151.27 (m), -154.11 – -154.48 (m), -159.96 – -160.46 (m). HRMS (ESI-TOF) m/z: $[M+Na]^+$ Calcd. for – $C_{25}H_{19}F_5N_6NaO_8S$ 681.0797; Found 681.0797.

(2R,3R,4R,5S)-5-(azidomethyl)-4-((N,N-dimethylsulfamoyl)methyl)-2-(2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)tetrahydrofuran-3-yl acetate 21: To a solution of **20b** (160 mg, 0.28 mmol) in acetonitrile (5 mL) was added dimethyl amine (560 µL, 0.56 mmol). The resulting mixture was stirred for 1h at room temperature and then concentrated *in vacuo* to give crude **21** which was purified by flash chromatography on silica gel using a gradient of 0% to 10% MeOH in DCM affording **21** as a white foam (120 mg, 0.28 mmol, quant).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.58 (s, 1H), 7.39 (d, J = 8.1 Hz, 1H), 5.78 (d, J = 8.1 Hz, 1H), 5.61 (s, 1H), 5.57 – 5.49 (m, 1H), 4.18 (dt, J = 7.2, 3.4 Hz, 1H), 3.83 (dd, J = 13.5, 2.8 Hz, 1H), 3.69 (dd, J = 13.5, 3.9 Hz, 1H), 3.29 – 3.15 (m, 2H), 2.98 – 2.92 (m, 1H), 2.92 (s, 6H), 2.16 (s, 3H); $^{13}C\{^{1}H\}$ NMR (101 MHz, $CDCl_3$) δ 169.7, 162.5, 149.8, 1401.0, 103.2, 92.0, 81.7, 76.8, 52.0, 44.5, 37.5, 37.4, 20.7. HRMS (ESI-TOF) m/z: $[M+Na]^+$ Calcd. for – $C_{14}H_{20}N_6NaO_7S$ 439.1006; Found 439.1008.

Sa-dinucleotide-compound **23**: To a solution of **21** (120 mg, 0.28 mmol) in MeOH (5 mL) was added 10% Pd/C (60 mg). The resulting suspension was stirred for 1 h at room temperature under an H_2 atmosphere (balloon). The resulting suspension was filtered over Celite and the filtrate was concentrated *in vacuo* to give crude amine **22**, which used in the next step without further purification. After dissolving crude **22** in DMF (2 mL). To the resulting solution was added PFP-ester **20a** (200 mg, 0.30 mmol) and DiPEA (80 µL, 0.56mmol). The resulting mixture was stirred for 4h at room temperature under a N_2 atmosphere followed by concentration *in vacuo* to give crude **23** which was purified by flash chromatography on silica gel using a gradient from 0% to 5% MeOH in DCM and the desired product was obtained as a white foam (150 mg, 0.17 mmol, 60%).

LC/MS: t_R – 5.39min, $[M]^+$ m/z – 888.92, 10 min run.

Sa-trinucleotide compound **24**: To a solution of dinucleotide **23** (140mg, 0.16 mmol) in MeOH (5 mL) was added 10% Pd/C (50 mg). The resulting suspension was stirred overnight at room temperature under a H_2 atmosphere (balloon). The resulting suspension was filtered over Celite and the filtrate was concentrated *in vacuo* to give the crude amino sa-dinucleotide product as a foam which used in the next step without further purification. After dissolving in DMF (5 mL) and addition of PFP-ester **20b** (177 mg, 0.32 mmol) and DiPEA (93 µL, 0.64mmol), the resulting mixture was heated to 70°C under microwave irradiation and stirred for 20 min. The reaction mixture was then concentrated *in vacuo* to give the crude **24** which was purified by flash chromatography on silica gel using a gradient from 0% to 20% MeOH in DCM and the desired product was obtained as a white foam (72 mg, 0.06 mol, 36%).

LC/MS: t_R – 5.31min, $[M]^+$ m/z – 1233.92, 10 min run.

Sa-tetranucleotide compound **26**: To a solution of **24** (72 mg, 58 µmol) in THF (5 mL) was added 10% Pd/C (50 mg). The resulting suspension was stirred overnight at room temperature under a H_2 atmosphere (balloon). Next, the resulting suspension was filtered over Celite and the filtrate was concentrated *in vacuo* to give the crude amino sa-trinucleotide product as a foam which used in the next step without further purification. After dissolution in DMF (2 mL), PFF-ester **20a** (82 mg, 120 µmol) and DiPEA (60 µL 350 µmol) were added. The mixture was heated to 85°C under microwave irradiation and stirred for 15 min. Next, the reaction mixture was concentrated *in vacuo* to give crude protected tetranucleoride **25**, which was dissolved in 7N ammonia in methanol. The resulting mixture was stirred overnight at room temperature. The reaction mixture was then concentrated *in vacuo* to give crude **26.** Crude **26** was purified by preparative HPLC to give >95% pure (by HPLC) **26** (5.4 mg, 4.1 µmol, 7% yield over two steps).

LC/MS: t_R – 9.07min, $[M]^+$ m/z – 1330.17, 40 min run.

HPLC: t_R – 5.85min.

(2R,3R,4R,5S)-5-(azidomethyl)-2-(4-benzamido-2-oxopyrimidin-1(2H)-yl)-4-((N-isobutylsulfamoyl)methyl)tetrahydrofuran-3-yl acetate 27: Compound **27** was synthesized

according to the procedure used for the preparation of **21**. Purification by flash chromatography on silica gel using a gradient from 0% to 5% MeOH in DCM gave the desired product **27** as a white foam (110 mg, 0.20 mmol, 87%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.91 (s, 1H), 7.94 (t, *J* = 7.3 Hz, 3H), 7.66 – 7.48 (m, 4H), 5.80 (d, *J* = 6.2 Hz, 1H), 5.68 (d, *J* = 1.7 Hz, 1H), 4.21 (dt, *J* = 8.5, 3.8 Hz, 1H), 3.82 (d, *J* = 3.9 Hz, 2H), 3.38 (dd, *J* = 14.4, 7.1 Hz, 1H), 3.32 – 3.19 (m, 1H), 3.01 (dd, *J* = 14.4, 5.4 Hz, 1H), 2.94 – 2.89 (m, 2H), 2.14 (s, 3H), 1.86 – 1.73 (m, 1H), 0.94 (s, 3H), 0.92 (s, 3H). ^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$) δ 169.8, 133.2, 129.00, 127.8, 93.9, 82.4, 77.5, 51.8, 50.6, 47.9, 38.0, 29.0, 20.8, 19.8, 19.8. HRMS (ESI-TOF) m/z: $[M+Na]^+$ Calcd. for – $C_{23}H_{29}N_7NaO_7S$ 570.1732; Found 570.1741.

Compound **29**: Compound **29** was synthesized according to the procedure used for the preparation of **22**. Purification by flash chromatography on silica gel using a gradient from 0% to 15% MeOH in DCM gave the desired product **22** as a white foam (120 mg, 0.12 mmol, 62%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 12.02 (s, 1H), 9.54 (s, 2H), 8.11 (s, 1H), 8.09 (s, 1H), 7.88 (s, 1H), 7.67 – 7.47 (m, 4H), 5.93 (s, 1H), 5.75 (s, 1H), 5.58 (d, *J* = 6.8 Hz, 1H), 4.24 – 4.07 (m, 2H), 3.73 (d, *J* = 13.3 Hz, 1H), 3.65 – 3.32 (m, 6H), 3.21 (d, *J* = 14.2 Hz, 1H), 3.08 (d, *J* = 12.8 Hz, 1H), 3.01 – 2.85 (m, 2H), 2.26 (s, 3H), 2.13 (s, 3H), 2.09 (s, 3H), 1.95 (s, 2H), 1.85 – 1.72 (m, 1H), 0.93 (s, 3H), 0.92 (s, 3H). ^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$) δ 170.3, 169.9, 147.2, 133.4, 129.00, 50.6, 29.0, 20.7, 19.9, 19.8. HRMS (ESI-TOF) m/z: $[M+Na]^+$ Calcd. for - $C_{38}H_{47}N_{13}NaO_{14}S_2$ 996.2699; Found 996.2678.

Compound **30**: Compound **30** was synthesized according to the procedure used for the preparation of **26**. Purification by preparative HPLC gave the desired product **30** as a white foam (20 mg, 27 μmol, 23%).

^{1}H NMR (400 MHz, Methanol-d_4) δ 8.22 (s, 2H), 8.20 (s, 1H), 6.08 (d, *J* = 7.9 Hz, 1H), 5.93 (s, 1H), 5.68 (s, 1H), 4.78 (d, *J* = 5.1 Hz, 1H), 4.61 – 4.54 (m, 1H), 4.31 – 4.23 (m, 1H), 4.22 – 4.15 (m, 1H), 3.87 (dd, *J* = 13.8, 2.8 Hz, 1H), 3.74 (dd, *J* = 13.8, 4.0 Hz, 1H), 3.70 – 3.57 (m, 2H), 3.52 – 3.42 (m, 2H), 3.29 – 3.24 (m, 1H), 3.12 (dd, *J* = 14.3, 4.5 Hz, 1H), 3.03 – 2.94 (m, 1H), 2.88 (d, *J* = 1.1 Hz,

1H), 2.86 (d, *J* = 1.0 Hz, 1H), 2.53 – 2.43 (m, 1H), 1.83 – 1.68 (m, 1H), 0.94 (s, 3H), 0.92 (s, 3H). $^{13}C\{^{1}H\}$ NMR (101 MHz, Methanol-d_4) δ 160.2, 157.2, 154.3, 147.5, 144.7, 136.3, 135.8, 128.5, 115.0, 94.1, 93.0, 91.3, 83.0, 81.9, 75.1, 75.0, 51.1, 50.0, 48.2, 48.1, 48.0, 47.9, 47.8, 47.7, 47.6, 47.5, 47.4, 47.2, 47.0, 46.6, 43.3, 38.6, 38.5, 28.9, 18.9. HRMS (ESI-TOF) m/z: $[M + H]^+$ Calcd. for $C_{25}H_{38}N_{13}O_{10}S_2$ 743.2284; Found: 743.2301.

isobutyl ((3aR,5S,6aR)-5-((R)-2,2-dimethyl-1,3-dioxolan-4-yl)-2,2-dimethylfuro[2,3-d][1,3]dioxol-6(3aH,5H,6aH)-ylidene)methanesulfonate 33: Diacetone-*D*-glucose **31** (45g, 170 mmol) was oxidized to the corresponding ketone **32** in an analogous procedure to that described for **12** to give crude ketone **32** (40 g 150 mmol) which was used in the next step without further purification. As described in literature (Blade *et al.*[21]). To a stirred solution of Horner–Wadsworth–Emmons reagent **13** (18g, 64 mmol) in dry THF (400 mL) kept under N_2 atmosphere was added NaH (2.6 g, 60% in mineral oil, 64 mmol) at 0°C. The resulting mixture was stirred for 30 min at 0°C after which a solution of crude ketone **32** (15 g, 58 mmol) in THF (100 mL) was added drop wise. The reaction mixture was kept at 0°C for 2h. Water (100 mL) was then added and the quenched reaction mixture was warmed to room temperature. THF was removed *in vacuo*, additional water (200 mL) was added and mixture was extracted with DCM (3 · 500mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated *in vacuo* to give crude **33** which was purified by flash chromatography on silica gel using a gradient of 0% to 40% EtOAc in hexanes and the desired product was obtained as a white solid as a 1:1 mixture of E/Z isomers (16 g, 41 mmol, 72%, the overall yield over two steps was 65%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 6.83 – 6.81 (m, 1H), 6.56 (t, *J* = 1.8 Hz, 1H), 5.96 (d, *J* = 4.8 Hz, 1H), 5.87 (d, *J* = 4.1 Hz, 1H), 5.68 (dt, *J* = 4.4, 1.3 Hz, 2H), 5.17 (d, *J* = 4.7 Hz, 1H), 4.66 (dt, *J* = 8.2, 1.5 Hz, 1H), 4.32 – 4.26 (m, 1H), 4.17 – 4.09 (m, 1H), 4.07 – 3.98 (m, 6H), 3.92 (dd, *J* = 9.3, 6.5 Hz, 1H), 3.77 (dd, *J* = 9.2, 7.8 Hz, 1H), 2.11 – 1.98 (m, 2H), 1.51 (s, 3H), 1.46 – 1.45 (m, 4H), 1.45 – 1.44 (m, 3H), 1.42 (s, 3H), 1.40 (s, 3H), 1.35 (s, 3H), 1.34 (s, 3H), 1.34 (s, 3H), 0.99 (s, 3H), 0.99 (d, *J* = 1.3 Hz, 4H), 0.98 (s, 3H), 0.97 (d, *J* = 1.2 Hz, 3H). $^{13}C\{^{1}H\}$ NMR (101 MHz, $CDCl_3$) δ 156.0, 154.5, 122.3, 122.0, 114.3, 113.3, 110.4, 109.5, 105.0, 103.7, 82.2, 79.7, 79.0, 78.7, 76.3, 67.5, 65.4, 28.2,

28.2, 28.0, 27.8, 27.3, 27.0, 26.8, 26.0, 25.6, 25.3, 18.7, 18.6, 18.6, 18.6. HRMS (ESI-TOF) m/z: $[M+Na]^+$ Calcd. for – $C_{17}H_{28}NaO_8S$ 415.1378; Found 415.1397.

isobutyl ((3aR,5S,6R,6aR)-5-((R)-2,2-dimethyl-1,3-dioxolan-4-yl)-2,2-dimethyltetrahydrofuro[2,3-d][1,3]dioxol-6-yl)methanesulfonate 34: To a stirred solution of **33** (34 g, 87 mmol) in EtOH (2 L) was added $NaBH_4$ (20 g, 520 mmol) over 10 min at 0°C. The resulting mixture was stirred for 2h at 0°C. Glacial AcOH (14 mL) was then added to quench the reaction mixture and the resulting mixture was stirred for 15 min at room temperature after which the solvent was removed *in vacuo*. The obtained solid was partitioned between a sat. $NaHCO_3$ solution (500 mL) and EtOAc (500 mL). The organic layer was washed with brine, dried over $MgSO_4$, filtered and concentrated *in vacuo* to give **34** (34 g, 87 mmol, quant).

^1H NMR (400 MHz, $CDCl_3$) δ 5.85 (d, J = 3.6 Hz, 1H), 4.85 (t, J = 4.1 Hz, 1H), 4.03 (dd, J = 6.5, 0.9 Hz, 2H), 3.97 – 3.86 (m, 2H), 3.76 – 3.64 (m, 1H), 3.58 (dd, J = 14.6, 9.9 Hz, 1H), 3.12 (dd, J = 14.6, 3.2 Hz, 1H), 2.64 (tdd, J = 9.9, 4.6, 3.2 Hz, 1H), 2.04 (dq, J = 13.4, 6.7 Hz, 1H), 1.83 (dd, J = 7.7, 5.0 Hz, 1H), 1.52 (s, 3H), 1.35 (s, 3H), 1.01 (d, J = 1.7 Hz, 3H), 0.99 (d, J = 1.7 Hz, 3H). $^{13}C\{^1H\}$ NMR (101 MHz, $CDCl_3$) δ 112.3, 110.0, 105.2, 80.3, 79.9, 77.9, 76.2, 68.3, 45.1, 44.6, 28.3, 26.8, 26.7, 26.4, 25.1, 18.7, 18.7. HRMS (ESI-TOF) m/z: $[M+Na]^+$ Calcd. for – $C_{17}H_{30}NaO_8S$ 417.1550; Found 417.1554.

isobutyl ((3aR,5S,6R,6aR)-5-(hydroxymethyl)-2,2-dimethyltetrahydrofuro[2,3-d][1,3]dioxol-6-yl)methanesulfonate 35: Compound **34** (34g, 86 mmol) was dissolved in a mixture of glacial acetic acid (160 mL), water (80 mL) and MeOH (80 mL), and then stirred overnight at 50°C. Next, the reaction mixture was concentrated *in vacuo* and co-evaporated twice with toluene. The obtained crude diol **35** was dissolved in MeOH (260 mL) and a solution of $NaIO_4$ (22 g, 100 mmol) in water (60mL) was added at room temperature. The resulting mixture was stirred for 15 min at room temperature, after which it was concentrated *in vacuo*. The resulting solid was suspended in water (200 mL) and extracted with $CHCl_3$ (3 · 400mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated *in vacuo*. The resulting crude aldehyde was dissolved in a mixture of MeOH (280 mL) water (70 mL) and $NaBH_4$ (2.7g, 100 mmol) was added over 10 min at 0°C. The

reaction mixture was warmed to room temperature and stirred for 30 min after which MeOH was evaporated and the resulting reaction mixture extracted three times with EtOAc (3 · 300 mL). The combined organic layers were washed with brine and dried over $MgSO_4$, filtered and concentrated in vacuo to give crude **23** which was purified by flash chromatography on silica gel using a 0% to 50% EtOAc gradient in hexanes. Desired Alcohol **36** was obtained as a white solid (15.0 g, 46 mmol, 54%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.85 (d, *J* = 3.6 Hz, 1H), 4.85 (t, *J* = 4.1 Hz, 1H), 4.03 (dd, *J* = 6.5, 0.9 Hz, 2H), 3.97 – 3.86 (m, 2H), 3.76 – 3.64 (m, 1H), 3.58 (dd, *J* = 14.6, 9.9 Hz, 1H), 3.12 (dd, *J* = 14.6, 3.2 Hz, 1H), 2.64 (tdd, *J* = 9.9, 4.6, 3.2 Hz, 1H), 2.04 (dq, *J* = 13.4, 6.7 Hz, 1H), 1.83 (dd, *J* = 7.7, 5.0 Hz, 1H), 1.52 (s, 3H), 1.35 (s, 3H), 1.01 (d, *J* = 1.7 Hz, 3H), 0.99 (d, *J* = 1.7 Hz, 3H). ^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$) δ 112.2, 104.9, 80.5, 80.2, 76.1, 61.3, 45.8, 39.6, 28.3, 26.7, 26.4, 18.7, 18.7. HRMS (ESI-TOF) m/z: $[M+Na]^+$ Calcd. for - $C_{13}H_{24}NaO_7S$ 347.1140; Found 347.1135.

((3aR,5S,6R,6aR)-6-((isobutoxysulfonyl)methyl)-2,2-dimethyltetrahydrofuro[2,3-d][1,3]dioxol-5-yl)methyl 4-oxopentanoate 37: To a solution of **36** (560 mg, 1.7 mmol) in DCM (70 mL) were added levulinic acid (400 mg, 3.5 mmol), EDC HCl (500 mg, 2.6 mmol), DMAP (100 mg, 0.85 mmol) and DiPEA (890 µL, 5.1 mmol). After stirring for 16h the reaction mixture was diluted with DCM (30 mL) and this organic layer was washed with 1 M HCl (20 mL), a saturated $NaHCO_3$ solution (20 mL) and brine (20 mL). After drying over $MgSO_4$, filtering and concentration *in vacuo* crude **37** was obtained which was purified by flash chromatography on silica gel using a gradient of 0% to 20% EtOAc in hexane affording the desired levulinic ester as a colorless oil (260mg, 0.61 mmol, 36%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.86 (d, *J* = 3.6 Hz, 1H), 4.84 (t, *J* = 4.1 Hz, 1H), 4.34 (dd, *J* = 12.4, 3.1 Hz, 1H), 4.25 (dd, *J* = 12.4, 4.7 Hz, 1H), 4.03 (d, *J* = 6.5 Hz, 2H), 4.02 – 3.97 (m, 1H), 3.57 (dd, *J* = 14.5, 10.5 Hz, 1H), 3.14 (dd, *J* = 14.4, 2.7 Hz, 1H), 2.79 – 2.74 (m, 2H), 2.64 – 2.60 (m, 2H), 2.51 – 2.42 (m, 1H), 2.19 (s, 3H), 2.11 – 1.98 (m, 1H), 1.52 (s, 3H), 1.35 (s, 3H); ^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$) δ 206.4, 172.5, 112.2, 104.9, 79.9, 77.7, 76.2, 63.0, 45.4, 40.9, 37.9, 29.8, 28.3, 27.8, 26.7,

26.3, 18.7, 18.6; HRMS (ESI-TOF) m/z: $[M+Na]^+$ Calcd. for - $C_{18}H_{30}NaO_9S$ 445.1503; Found 445.1500.

(2R,3R,4R,5S)-4-((isobutoxysulfonyl)methyl)-5-(((4-oxopentanoyl)oxy)methyl)tetrahydrofuran-2,3-diyl diacetate 38: Compound **38** was prepared analogously to the procedure used for the synthesis of **16**. Purification by flash chromatography on silica gel using a gradient of 0% to 20% EtOAc in hexanes gave the desired product **38** as a white solid (250 mg, 0.54 mmol, 89%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 6.13 (s, 1H), 5.32 (d, *J* = 4.8 Hz, 1H), 4.29 – 4.19 (m, 3H), 4.03 (d, *J* = 6.6 Hz, 2H), 3.45 (dd, *J* = 14.4, 9.8 Hz, 1H), 3.28 (dd, *J* = 14.4, 4.3 Hz, 1H), 2.92 (tt, *J* = 9.3, 4.5 Hz, 1H), 2.80 – 2.75 (m, *J* = 6.4 Hz, 2H), 2.64 – 2.57 (m, *J* = 9.7, 3.7 Hz, 2H), 2.19 (s, 3H), 2.14 (s, 3H), 2.10 (s, 3H), 1.00 (d, *J* = 1.3 Hz, 3H), 0.98 (d, *J* = 1.3 Hz, 3H); ^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$) δ 206.3, 172.4, 169.3, 168.9, 98.3, 81.1, 76.3, 75.9, 65.3, 46.2, 37.8, 37.8, 29.8, 28.3, 27.7, 21.1, 20.6, 18.6. HRMS (ESI-TOF) m/z: $[M+Na]^+$ Calcd. for – $C_{19}H_{30}NaO_{11}S$ 489.1398; Found 489.1398.

((2S,3R,4R,5R)-4-acetoxy-3-((isobutoxysulfonyl)methyl)-5-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)tetrahydrofuran-2-yl)methyl 4-oxopentanoate 39: Compound **39** was prepared analogously to the procedure used for the synthesis of **17b**. Purification by flash chromatography on silica gel using a 0% to 80% gradient of EtOAc in hexane gave the desired product **39** as a white foam (150 mg, 0.29 mmol, 89%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.19 (s, 1H), 7.26 (s, 2H), 7.19 (d, *J* = 1.2 Hz, 1H), 5.59 (d, *J* = 2.2 Hz, 1H), 5.52 (dd, *J* = 6.8, 2.2 Hz, 1H), 4.43 (t, *J* = 3.6 Hz, 2H), 4.15 (dt, *J* = 9.6, 3.4 Hz, 1H), 4.02 (d, *J* = 6.6 Hz, 2H), 3.46 (dd, *J* = 14.4, 9.3 Hz, 1H), 3.25 (dd, *J* = 14.4, 4.6 Hz, 1H), 3.20 – 3.13 (m, 1H), 2.85 – 2.77 (m, 2H), 2.61 (td, *J* = 6.6, 4.8 Hz, 2H), 2.20 (s, 3H), 2.16 (s, 3H), 2.08 – 1.99 (m, 1H), 1.93 (d, *J* = 1.2 Hz, 3H), 0.99 (s, 3H), 0.98 (s, 3H); ^{13}C{^{1}H} NMR (126 MHz, $CDCl_3$) δ 206.6, 172.6, 169.6, 163.2, 149.6, 137.0, 111.5, 92.5, 80.6, 76.4, 76.1, 62.9, 46.2, 38.0, 37.3, 29.7, 28.3, 27.8, 20.7, 18.6, 12.4. HRMS (ESI-TOF) m/z: $[M+Na]^+$ Calcd. for - $C_{22}H_{32}N_2NaO_{11}S$ 555.1619; Found 555.1629.

((2S,3R,4R,5R)-4-acetoxy-5-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-3-(((perfluorophenoxy)sulfonyl)methyl)tetrahydrofuran-2-yl)methyl 4-oxopentanoate 40:

Compound **40** was prepared analogously to the procedure used for the synthesis of **20b** and gave the desired PFP ester **40** as a white foam (180 mg,0.28 mmol, 88%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.49 (s, 1H), 7.21 (d, *J* = 1.2 Hz, 1H), 5.62 – 5.57 (m, 2H), 4.51 (dd, *J* = 12.6, 4.1 Hz, 1H), 4.41 (dd, *J* = 12.6, 3.0 Hz, 1H), 4.17 (dt, *J* = 9.9, 3.5 Hz, 1H), 3.86 (dd, *J* = 14.5, 10.1 Hz, 1H), 3.71 (dd, *J* = 14.4, 4.0 Hz, 1H), 3.44 – 3.32 (m, 1H), 2.90 – 2.72 (m, 2H), 2.68 – 2.51 (m, 2H), 2.17 (s, 3H), 2.16 (s, 3H), 1.92 (d, *J* = 1.1 Hz, 3H). ^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$) δ 206.7, 172.7, 169.5, 163.1, 149.6, 137.2, 111.7, 93.1, 80.5, 76.3, 62.5, 48.5, 38.0, 37.5, 29.6, 27.8, 20.5, 12.3. ^{19}F NMR (376 MHz, CDCl) δ -150.25 – -151.47 (m), -154.25 – -154.75 (m), -160.16 – -160.68 (m). HRMS (ESI-TOF) m/z: $[M+Na]^+$ Calcd. for – $C_{24}H_{23}F_5N_2NaO_{11}S$ 665.0822; Found 665.0835.

((2S,3R,4R,5R)-4-acetoxy-3-((N-(((2R,3S,5R)-3-((tert-butyldimethylsilyl)oxy)-5-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)tetrahydrofuran-2-yl)methyl)sulfamoyl)methyl)-5-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)tetrahydrofuran-2-yl)methyl 4-oxopentanoate 41: To a solution of **40** (32 mg, 50 µmol) in DMF (2 mL) was added 5'-amino 3'-TBDMS protected deoxy-thymidine derivative[31] (37 mg, 100 µmol) and DiPEA (18 µL, 100 µmol). The resulting mixture was heated to 60°C by microwave irradiation and stirred for 20 min. After concentration of the reaction mixture *in vacuo* crude sulfonamide **41** was obtained which was purified by flash chromatography on silica gel using a 0% to 5% gradient of MeOH in DCM and the desired sulfonamide dinucleotide **41** was obtained as a white foam (34 mg, 37 µmol, 74%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 9.53 (s, 1H), 9.41 (s, 1H), 7.18 – 7.12 (m, 1H), 7.07 (d, *J* = 1.4 Hz, 1H), 6.27 (s, 1H), 5.97 (t, *J* = 7.0 Hz, 1H), 5.57 (dd, *J* = 6.8, 2.1 Hz, 1H), 5.33 (d, *J* = 2.1 Hz, 1H), 4.44 (d, *J* = 3.8 Hz, 2H), 4.39 (dt, *J* = 6.6, 3.3 Hz, 1H), 4.15 (dt, *J* = 8.2, 3.8 Hz, 1H), 3.98 (q, *J* = 4.6 Hz, 1H), 3.45 – 3.26 (m, 4H), 3.18 (dd, *J* = 14.1, 4.0 Hz, 1H), 2.81 – 2.75 (m, 2H), 2.62 – 2.56 (m, 2H), 2.18 (s, 3H), 2.16 (s, 3H), 1.91 (d, *J* = 1.2 Hz, 3H), 1.90 (d, *J* = 1.2 Hz, 3H), 0.89 (s, 9H), 0.09 (s, 3H), 0.09 (s, 3H). ^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$) δ 207.2, 172.8, 170.3, 163.8, 163.5, 150.8, 150.4, 138.3, 137.6, 111.6, 111.4, 95.3, 86.1, 81.4, 72.8, 63.9, 48.0, 44.7, 39.2, 37.9, 37.6, 29.8, 27.8, 25.7,

20.8, 17.9, 12.4, 12.3, -4.7, -4.8. HRMS (ESI-TOF) m/z: $[M+Na]^+$ Calcd. for – $C_{34}H_{51}N_5NaO_{14}SSi$ 836.2819; Found 836.2815.

(2R,3R,4R,5S)-4-((N-(((2R,3S,5R)-3-((tert-butyldimethylsilyl)oxy)-5-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)tetrahydrofuran-2-yl)methyl)sulfamoyl)methyl)-5-(hydroxymethyl)-2-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)tetrahydrofuran-3-yl acetate 42: To a stirring solution of **41** (430 mg 0.53 mmol) in pyridine was added a 1 M solution of hydrazine hydrate in a 3:2 mixture of pyridine and acetic acid (5.3 mL). The reaction mixture was stirred for 10 min followed by concentration *in vacuo*. The residue was then redissolved in DCM and this solution was washed with 1 M HCl, a saturated $NaHCO_3$ solution and water. After drying over $MgSO_4$, filtration and concentration *in vacuo* crude **42** was obtained, which was purified by flash chromatography on silica gel using 0% to 10% MeOH gradient in DCM affording the desired compound as a white solid (300 mg, 0.42 mmol, 79%).

^1H NMR (400 MHz, $CDCl_3$) δ 9.95 (s, 0H), 10.05 – 9.79 (m, 1H), 7.29 (s, 1H), 7.12 (d, J = 1.5 Hz, 1H), 6.63 (s, 1H), 6.04 (t, J = 6.9 Hz, 1H), 5.66 – 5.58 (m, 1H), 5.35 (s, 1H), 4.37 (dt, J = 7.1, 3.9 Hz, 1H), 4.08 – 3.98 (m, 2H), 3.96 (dd, J = 6.5, 3.3 Hz, 1H), 3.85 (d, J = 12.7 Hz, 1H), 3.47 – 3.28 (m, 5H), 3.10 (d, J = 11.4 Hz, 1H), 2.51 – 2.37 (m, 1H), 2.24 – 2.15 (m, 1H), 2.16 (s, 3H), 1.85 (d, J = 1.0 Hz, 3H), 1.80 (s, 3H), 0.88 (s, 9H), 0.09 (s, 3H), 0.08 (s, 3H). $^{13}C\{^1H\}$ NMR (101 MHz, $CDCl_3$) δ 170.3, 164.2, 163.9, 151.0, 150.6, 138.5, 137.5, 111.5, 110.9, 85.8, 84.2, 77.8, 72.4, 60.8, 53.4, 47.5, 44.3, 39.3, 35.9, 25.7, 20.8, 17.9, 12.3, 12.2, -4.7, -4.8. HRMS (ESI-TOF) m/z: $[M+Na]^+$ Calcd. for – $C_{29}H_{45}N_5NaO_{12}SSi$ 738.2424; Found 738.2447.

(2R,3R,4R,5S)-5-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-4-((N-(((2R,3S,5R)-3-((tert-butyldimethylsilyl)oxy)-5-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)tetrahydrofuran-2-yl)methyl)sulfamoyl)methyl)-2-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)tetrahydrofuran-3-yl acetate 43: A solution of **42** (300 mg 0.41 mmol) in pyridine (3 mL), to which DMTrCl (170mg 0.50 mmol) was added, was stirred for 4h. After removal of the solvent in vacuo the crude compound **43** was purified by flash chromatography on silica gel using a gradient of

0% to 80% EtOAc in hexanes containing 0.1% TEA and gave the desired product as a yellowish solid (310mg, 0.30mmol, 72%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 9.18 (s, 1H), 7.47 – 7.39 (m, 2H), 7.35 – 7.17 (m, 8H), 7.01 (d, *J* = 1.4 Hz, 1H), 6.83 (d, *J* = 1.3 Hz, 2H), 6.81 (d, *J* = 1.3 Hz, 2H), 5.93 (s, 1H), 5.83 (t, *J* = 7.1 Hz, 1H), 5.70 – 5.64 (m, 1H), 5.55 (d, *J* = 2.4 Hz, 1H), 4.34 (dt, *J* = 6.4, 3.1 Hz, 1H), 4.10 (t, *J* = 4.3 Hz, 1H), 3.93 (dt, *J* = 6.9, 3.7 Hz, 1H), 3.77 (s, 5H), 3.46 (qd, *J* = 10.8, 3.7 Hz, 2H), 3.41 – 3.31 (m, 2H), 3.23 (q, *J* = 5.9 Hz, 2H), 3.07 (d, *J* = 10.0 Hz, 1H), 2.60 – 2.48 (m, 1H), 2.15 (s, 3H), 2.14 – 2.09 (m, 1H), 1.85 (d, *J* = 1.1 Hz, 3H), 1.64 (d, *J* = 1.1 Hz, 3H), 0.88 (s, 9H), 0.08 (s, 6H). ^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$) δ 169.9, 163.6, 163.4, 158.7, 158.6, 150.5, 150.3, 144.3, 138.1, 137.2, 135.3, 130.2, 130.1, 128.2, 128.0, 127.1, 113.3, 111.6, 111.4, 87.0, 85.9, 82.2, 72.8, 63.7, 55.2, 53.4, 48.3, 44.4, 39.0, 38.0, 25.8, 20.8, 17.9, 12.3, 12.1, -4.7, -4.8. HRMS (ESI-TOF) m/z: $[M+Na]^+$ Calcd. for – $C_{50}H_{63}N_5NaO_{14}SSi$ 1040.3712; Found 1040.3754.

(2R,3R,4R,5S)-5-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-4-((N-(((2R,3S,5R)-3-hydroxy-5-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)tetrahydrofuran-2-yl)methyl)sulfamoyl)methyl)-2-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)tetrahydrofuran-3-yl acetate 44: A solution of **43** (310 mg 0.30 mmol) in THF (10 mL), to which 1M TBAF in THF (450 µL 0.46 mmol) was added, was stirred for 1h. After removal of the solvent in vacuo the crude compound **44** was purified by flash chromatography on silica gel using a gradient of 0% to 80% EtOAc in hexanes containing 0.1% TEA and gave the desired product as a yellowish solid (260mg, 0.29mmol, 96%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44 – 7.38 (m, 2H), 7.36 – 7.22 (m, 9H), 7.21 – 7.14 (m, 1H), 6.80 (dd, *J* = 9.0, 2.8 Hz, 4H), 6.11 (t, *J* = 6.8 Hz, 1H), 5.78 – 5.70 (m, 1H), 5.42 (s, 1H), 4.45 (s, 1H), 4.05 (d, *J* = 6.9 Hz, 1H), 3.92 (d, *J* = 4.6 Hz, 1H), 3.76 (d, *J* = 2.8 Hz, 0H), 3.73 (d, *J* = 0.9 Hz, 6H), 3.50 – 3.32 (m, 3H), 3.20 (d, *J* = 5.6 Hz, 2H), 3.02 (d, *J* = 10.7 Hz, 1H), 2.85 (q, *J* = 7.3 Hz, 2H), 2.14 (s, 3H), 1.84 (s, 3H), 1.67 (s, 3H), 1.21 (t, *J* = 7.2 Hz, 3H). ^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$) δ 169.9, 163.6, 163.4, 158.7, 158.7, 150.5, 150.3, 144.3, 138.1, 137.2, 135.3, 130.2, 130.1, 128.2, 128.0, 127.1, 113.3, 111.6, 111.4, 87.0, 85.9, 82.2, 72.8, 63.7, 55.2, 53.0, 48.3, 44.4, 39.0, 38.1, 25.7, 20.8, 17.9,

12.3, 12.1, -4.7, -4.8. HRMS (ESI-TOF) m/z: $[M+Na]^+$ Calcd. for – $C_{44}H_{49}N_5NaO_{14}S$ 926.2847; Found 926.2889.

(2R,3R,4R,5S)-5-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-4-((N-(((2R,3S,5R)-3-(((2-cyanoethoxy)(diisopropylamino)phosphino)oxy)-5-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)tetrahydrofuran-2-yl)methyl)sulfamoyl)methyl)-2-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)tetrahydrofuran-3-yl acetate 45: To a stirred solution of **44** (260 mg, 0.29 mmol) in dry DCM (5 mL) was added 2-Cyanoethyl *N,N*-diisopropylchlorophosphoramidite (260 mg 0.37 mmol) and DiPEA (130 µL 0.75 mmol) After stirring for 3 h, the solvent was removed in vacuo and the crude compound **45** was purified by flash chromatography on silica gel using a gradient of 0% to 10% MeOH in DCM containing 0.1% TEA and the desired amidite was obtained as a white solid (180 mg, 0.16 mmol, 55%).

^{31}P NMR (162 MHz, $CDCl_3$) δ 149.58 (s), 148.33 (s). HRMS (ESI-TOF) m/z: $[M+Na]^+$ Calcd. for – $C_{53}H_{66}N_7NaO_{15}PS$ 1126.3931; Found 1126.3967.

Synthesis and purification of sulfonamide containing antisense oligodeoxynucleotide (DNA-SaASO 33)

DNA-SaRNA **46** was synthesized using standard solid phase oligonucleotide synthesis protocols on an ABI 394 synthesizer. Phosphoramidites and CPG supports loaded with standard nucleosides were purchased from LINK Technologies Ltd (Bellshill, UK). For the modified phosphoramidite **45** four coupling cycles with 10 minutes coupling time each were used. DNA-SaRNA **46** was cleaved from the CPG support overnight by shaking with 1.5 mL of ammonia at 60 °C. The CPG was washed with water and the combined liquid fractions were freeze-dried. DNA-SaRNA **46** was dissolved in water and filtered before purification by reverse-phase HPLC on a Dionex UltiMate 3000 System. The purified DNA-SaRNA **33** was freeze dried and the DMT protection group was removed by shaking with 1 ml of 80% AcOH for an hour. After desalting and freeze-drying, pure DNA-SaRNA **46** was obtained as a white solid (198 nmol, 19.8%).

*Analytical Data of **46***

MALDI-ToF mass spectra were recorded using a Shimadzu Biotech Axima CFR spectrometer, using 3-hydroxypicolinic acid (HPA) as the matrix. All mass spectra were recorded in negative mode (Fig S1-4). Analytical RP-HPLC was performed on a Dionex UltiMate 3000 system using a Clarity 5 µM Oligo-RP column (250 x 4.6 mm) in a triethylammonium acetate (TEAA) Buffer system. (Buffer A: 100 mM TEAA pH 7.5 in water. Buffer B 100 mM TEAA pH 7.5 in 80% MeCN. Flow rate: 1 mL/min)

DNA-SaASO 46: MS: $[M-H]^-$ calcd. 3962.7 found 3963.3. HPLC: retention time 12.0 min

Determination of melting curves by thermal UV-Vis measurements

Complementary oligonucleotides were diluted to 3 µM each in 10 mM Cacodylate buffer (10 mM sodium cacodylate, 10 mM KCl, 10 mM $MgCl_2$, 5 mM $CaCl_2$) previously set to the desired pH. UV measurements were obtained using a Shimadzu UV-1800 with an 8-series micro multi cell, each cell containing a sample volume of 100 µL. The UV spectra were obtained over a range from 20 to 90 °C for ODN1 using a ramp speed of 1 °C per minute and measurement intervals of 0.4 °C. The measured wavelength was 260 nm. All measurements were performed 3 times to obtain an average value.

Supporting information

^{1}H, ^{13}C, ^{19}F and ^{31}P NMR spectra as well as HPLC and LC-MS traces

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Notes

The authors declare no competing financial interest

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